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UNRESPONSIVENESS OF THE LEOPARD FROG *RANA PIPIENS* AS A TEST ANIMAL FOR BOVINE PREGNANCY

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The purpose of this research was to determine if the male of the common American frog *Rana pipiens* could be used as a test animal for bovine pregnancy.

Ever since Galli Mainini (1947) and Wiltberger and Miller (1948) first introduced the use of the male toad and the male frog respectively as test animals for the detection of early human pregnancy, there has been a considerable interest in the possible application of a similar technique for diagnosing early bovine pregnancy.

Cowie (1948) stated that many investigators have tried and failed to find gonadotropins in the urine and blood serum of pregnant cows by using rats, mice and rabbits as test animals, and that even ether-extracted pregnant cow's urine failed to provoke a positive reaction in female rabbits. Other investigators failed to find gonadotropins in the bovine placenta, endometrium, amniotic fluid, milk and saliva. Cowie concludes that if gonadotropins exist in the blood and urine of pregnant cows they must be in a form that cannot be detected by the usual biological methods.

Bhaduri (1950) claimed that bovine pregnancy as early as the 22nd day after breeding may be detected by using the male toad, *Bufo melanostictus*, as a test animal. This test is based on what Bhaduri calls "filter paper dialysis of hormone extraction from faeces." He injects the toad subcutaneously with an initial dose of 10 cc of this "dialysed" solution. If positive results (emission of spermatozoa) are not obtained within one hour, a second and, if necessary, a third injection of 5 cc each are made. No sperm emission after the third injection means a negative test.

Nadal and Biaggi (1950) obtained negative results when the male toads, *Bufo marinus*, were injected with pregnant cows' urine and blood serum separately. Although the dose of urine was only 5 cc some of the toads died within an hour after the injection. The cows ranged in gestation from 79 to 180 days.

PROCEDURE

In order to avoid false positive reactions caused by the injection of an excessive amount of liquid, whether any gonadotropin is present in it or not, the authors decided to inject not more than 7 cc at a time, but 3 cc and 5 cc doses were also

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tried. Giltz and Miller (1950) stated that 10 cc doses of distilled water provoked sperm emmision in 18 out of 30 male *Rana pipiens* frogs tested. (Hereafter "frog" is used to mean male frog *R. pipiens*.)

To test if the amount of 7 cc would cause a false positive reaction when injected because of volume alone, four frogs were injected with 7 cc of distilled water each and four more with 7 cc of physiological saline solution each. All frogs were tested for presence of sperm in urine samples immediately before injection. All of these frogs gave negative results when tested twice at one-hour intervals after injections were made.

First test. Four non-pregnant and six pregnant cows were selected from The Ohio State University dairy herd. Pregnancy was determined by last date of service and subsequent rectal palpation by experienced members of the staff of the College of Veterinary Medicine. Urine-free fecal material was obtained manually from each cow's rectum. Eighty grams of feces from each cow were placed in individual collodion dialysis bags and each bag tied and placed in a

TABLE 1

Results of injections of dialysed fecal solutions using collodion bags

Dose	Non-Pregnant Cows			Pregnant Cows		
	Reactions of Frogs			Reactions of Frogs		
	−−	−+	++	−−	−+	++
3 cc	4	0	0	6	0	0
5 cc	3	1	0	6	0	0
7 cc	2	0	2	6	0	0

TABLE 2

Results of injections of concentrated dialysed fecal solutions

Dose	Non-Pregnant Cows		Pregnant Cows	
	Reactions of Frogs		Reactions of Frogs	
	−	+	−	+
3 cc	3	1	4	2

graduated cylinder containing 130 cc of distilled water. All ten cylinders were placed in a domestic refrigerator for 24 hours. This dialysed solution was then filtered. Injections were made into six frogs for each cow, two frogs with 3 cc, two frogs with 5 cc, and two frogs with 7 cc, at one hour intervals. The reactions of the frogs to these injections of fecal solutions from non-pregnant and pregnant cows are presented in table 1.

In table 1, the double-minus sign (− −) is used when both frogs injected with the same solutions gave negative reactions. The minus-plus sign (− +) indicates one frog giving a negative reaction, the other giving a positive reaction. When the double-plus sign (+ +) is used, it indicates that both frogs gave a positive reaction.

Second test. These same solutions were then concentrated separately using the kaolin suspension method for hormone extraction. One frog per cow was injected with 3 cc of this solution. The results of this test are presented in table 2.

Table 2 shows that the injection of 3 cc of the concentrated fecal solution of

four non-pregnant cows into four frogs resulted in three negative and one positive reaction. The results of injection of fecal solutions from six pregnant cows into six frogs show four negative and two positive reactions.

Third test. Suspecting that because of its probable size the gonadotropic hormone molecule, if present in the feces, might not be able to pass through the pores of the collodion bag, it was decided to run another test using muslin bags.

For this purpose fecal samples were obtained from five non-pregnant and ten pregnant cows. In this case 50 grams of each cow's feces were placed in a muslin bag and each bag immersed in 150 cc of distilled water. Samples of these solutions for injections were taken after 24 hours and after 48 hours subsequent to the immersion of the feces-filled bags in water. Only 7 cc doses were used this time in each of two frogs per cow tested. The results of this test are presented in table 3.

Table 3 shows the results of injection of filtered fecal solutions of five non-pregnant and ten pregnant cows into frogs in 7 cc dosages. The reactions of the two injected frogs per cow are indicated in the same manner as indicated in table 1.

All frogs were tested for presence of sperm in their urine immediately before being injected and twice at one-hour intervals after injections were made.

TABLE 3

Results of injections of filtered fecal solutions using muslin bags

	NON-PREGNANT COWS						PREGNANT COWS					
	After 24-hr. filtration Reactions of Frogs			After 48-hr. filtration Reactions of Frogs			After 24-hr. filtration Reactions of Frogs			After 48-hr. filtration Reactions of Frogs		
	—	−+	++	—	−+	++	—	−+	++	—	−+	++
7 cc	5	0	0	5	0	0	8	1	1	8	2	0

RESULTS AND DISCUSSION

From the results of this and previous investigations concerning the use of various test animals to diagnose early bovine pregnancy, it appears that the pregnant cow does not liberate a gonadotropic hormone of placental origin in its body fluids nor in the feces, or that if the hormone is liberated it is in such a low concentration or of such a character that the various test animals, including the male frog, do not react to it.

These results do not agree with those obtained by Bhaduri (1950), who used a different genus of amphibian, *Bufo melanostictus*, as his test animal. Such a difference in the responsiveness to the gonadotropic hormone produced by the pregnant cow between these two amphibians is unexpected.

In the experiment with *Rana pipiens*, the author used a greater concentration of feces (in grams of feces per cc of water) and also dialysed the feces for a longer period than did Bhaduri. Concentration of the fecal solution as explained under the second test did not seem to have any effect on the frog reaction to it. In all cases where positive reactions were obtained, a small number of sperm were observed. None of the frogs used in this work died as a result of the injection of the fecal solution. No satisfactory reason can be given at the present for the sporadic positive reactions observed in tables 1, 2 and 3.

The data shown in the tables were treated statistically using Fisher's exact method for finding the probability in a 2×2 table when n is very small. In table 1, none of the injected fecal solutions from pregnant cows elicited a positive reaction. A complete explanation for the false positive results observed in case of non-pregnant cows is not attempted at this time.

In table 2, the deviation between the observed and expected was only 0.2 which means that the probability of deviations as great as or greater could occur through chance alone in 50 percent of the cases. With a probability of this size the deviation was considered statistically insignificant.

In table 3, it is shown that none of the non-pregnant cows gave a positive reaction. In the case of the pregnant cows, the probability of getting a deviation as great as or greater than the observed through chance alone is also of such magnitude that the deviation was considered statistically insignificant. These results support the hypothesis that there is no difference between the reactions to fecal solutions from pregnant and non-pregnant cows.

SUMMARY AND CONCLUSIONS

Fecal samples of 23 cows, 14 of them pregnant, stages of gestation ranging from 27 to 193 days, of The Ohio State University dairy herd were placed in bags, some collodion and some muslin, and submerged in distilled water for 24 and 48 hours. The dialysed solutions were injected subcutaneously in adult male frogs, *Rana pipiens*, in 3 cc, 5 cc and 7 cc doses at one-hour intervals. Also concentrated dialysed fecal solution in 3 cc doses was similarly injected into other frogs. Frogs' urine was then examined under the microscope. Urine samples showing no sperm two hours after the last injection were called negative.

Samples of two non-pregnant cows produced a positive frog reaction (spermatozoa were observed in injected frogs' urine), while most of the pregnant cows produced negative results.

No appreciable difference in the results was observed when using the collodion or muslin bag to obtain the fecal solution. Also, no difference was observed in the cases when the concentrated fecal solution was injected.

From the results of this work one or more of the following conclusions may be drawn: (1) the pregnant cow probably does not liberate any gonadotropic hormone in the feces; (2) the gonadotropic hormone in the feces of the pregnant cow, if present, must be in such a low titre that the male frog will not react to it; (3) the male frog is not a suitable test animal in diagnosing bovine pregnancy with the techniques commonly employed.

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PREPARATION OF IRON SOLUTIONS FREE FROM CHLORIDE FOR TITRATION WITH PERMANGANATE[1, 2]

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It is commonly recognized that the most accurate results in the titration of ferrous iron with permanganate are obtained when chloride is not present. Since hydrochloric acid is the best solvent for iron ores and certain other materials containing iron, the chloride must ordinarily be removed in some way in order to obtain such results. It may be removed, as suggested by Willard (1912), by evaporating the solution of the sample with an excess of perchloric acid, but this procedure does not appear to have been commonly adopted. One disadvantage of this procedure is that the evaporation must be conducted with some care in order to avoid removal of all the excess of perchloric acid, as this may lead to decomposition of the ferric perchlorate. Another disadvantage is that with ores or other materials containing unsuspected organic matter there is a possibility of mechanical loss from violent reaction. The usual procedure is to evaporate the solution of the sample with an excess of sulfuric acid. The advantages, aside from the minor one of low cost, are that no special attention is required during evaporation and that there is no such possibility of mechanical loss. However, it does have a serious disadvantage that the iron sulfate so obtained is usually difficult to dissolve, especially when the residue has been heated with the concentrated sulfuric acid at a high temperature for an unusually long time. Even with careful technique it may take as long as half an hour to bring the iron sulfate completely into solution on adding the proper amount of water and heating. With careless technique the time required may be much longer (Willard and Furman, 1940). Another difficulty arises if the sample contains silica. Some of this goes into colloidal suspension in the hydrochloric acid, becomes dehydrated during the evaporation with the sulfuric acid, and appears as an insoluble residue that is very similar in color and general appearance to the iron sulfate, so that it is not easy to decide when all the difficultly soluble iron sulfate has been dissolved. The primary purpose of this investigation was to study methods for dissolving the iron sulfate obtained by evaporating an iron solution containing chloride with sulfuric acid. An incidental purpose was to determine the composition of this iron sulfate, about which some uncertainty appears to exist.

COMPOSITION OF THE SALT

Some investigators have stated or suggested, but without citing experimental evidence, that the iron sulfate obtained by evaporating iron chloride solutions with hot concentrated sulfuric acid is difficultly soluble in water or dilute sulfuric acid solution because it is a basic salt, or a mixture of basic salts of indefinite composition. That such a basic salt or mixture of basic salts could be formed and exist in the presence of an excess of sulfuric acid seems unlikely, but not unlikely that such could be formed if all the free sulfuric acid were removed by evaporation and the dry residue were further heated at an elevated temperature. However, from the standpoint of normal analytical procedure the composition of the salt produced in this latter way is of small importance. To settle this question about its composition, analyses were made of specimens of the salt isolated by different methods.

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A solution of pure ferric chloride in dilute hydrochloric acid, to which had been added an excess of concentrated sulfuric acid, was evaporated until hydrogen chloride was no longer expelled, and the mixture of the residual sulfate and concentrated sulfuric acid was heated at the decomposition temperature of this acid for five minutes longer. After cooling to room temperature, the salt was isolated from the mixture by these two methods:

I. By the use of a porcelain filter crucible most of the sulfuric acid was separated from the salt. The remainder of the acid was removed by washing with 95 per cent ethyl alcohol, and the alcohol by washing with ethyl ether. To make sure that all the sulfuric acid and solvents had been removed and to stimulate somewhat extreme conditions of treatment from the standpoint of analytical procedure, the salt was then heated to constant weight at 300° C in a muffle. The product was yellowish white and noticeably crystalline.

II. Most of the sulfuric acid was removed by the use of a filter crucible, but the remainder was removed directly by heating at 300° C to constant weight. This product was also crystalline but had an orange tint.

The method of analyzing these products was to heat weighed samples at 800° C to constant weight. Their iron content in terms of ferric oxide was given directly by the weight of ferric oxide thus obtained. Their sulfur content in terms of sulfur trioxide was found by difference. The results are shown in table 1.

TABLE 1

Analyses of Samples of Iron Sulfate

Method of Preparation	Samples Taken g.	Fe_2O_3 Found g.	Fe_2O_3 Content %	SO_3 Content %	Ratio of Fe_2O_3 to SO_3
I	2.1191	0.8444	39.85	60.15	0.332
	0.8142	0.3246	39.87	60.13	0.332
II	2.6870	1.0692	39.79	60.21	0.331
	0.6960	0.2764	39.71	60.29	0.330
Calculated for $Fe_2(SO_4)_3$:			39.94	60.06	0.333

From these results it is evident that the salt, when isolated by the methods described, is simply anhydrous normal ferric sulfate. In spite of the difference in the color of the products prepared by the two methods, no significant difference in composition is indicated by the analyses. The reason for this difference in color was not investigated further. The possibility still remained that the difficultly soluble sulfate formed in the presence of a large excess of sulfuric acid is a complex salt containing additional combined sulfuric acid, similar in composition to the very slightly soluble complex salt formed when chromic chloride solutions are evaporated with an excess of sulfuric acid, and that the treatment at 300° C in the absence of sulfuric acid had decomposed this salt. To test this possibility some of the sulfate isolated by filtration and washing was dried at room temperature and at 110° C. Analyses of this product indicated that it had the same composition as the others. All the experiments therefore showed that anhydrous normal ferric sulfate is the only salt formed when ferric chloride solutions are evaporated with an excess of sulfuric acid.

METHODS OF DISSOLVING THE SULFATE

Experiments were made both on isolated samples of the sulfate, prepared by the methods just described, and on mixtures of the salt and sulfuric acid such as would be obtained in the usual procedure for the determination of iron.

According to the personal knowledge of the senior author, some analysts add finely divided zinc to the dilute sulfuric acid solution containing the ferric sulfate in suspension in order to increase the rate at which the salt dissolves. He has used this procedure himself and has observed many students using it under his direction. Under apparently similar conditions, the time required to dissolve the salt varies considerably, and sometimes for no apparent reason the procedure appears to be of little help. As no published account of this procedure could be located, it was systematically studied to determine under what conditions it would give satisfactory results.

In a typical group of experiments, ferric chloride solutions, each containing 0.100 g. of iron, were evaporated down with different volumes of concentrated sulfuric acid on a steam plate until no further reduction in volume was observed. Next, the residual solutions were evaporated on an electric hot plate for 30 min. at a temperature such that fumes of sulfur trioxide were continually evolved. After cooling, different volumes of water were added to the samples along with different weights of 20-mesh granulated zinc, and the mixtures were heated on steam plate and stirred at regular intervals to the same extent until all the ferric sulfate had dissolved. The results of many such experiments are summarized in table 2. The figures for the times in the last column are average figures, often

TABLE 2

Effect of Metallic Zinc in Accelerating the Rate of Solution of Anhydrous Ferric Sulfate

Series No.	Volume of H_2SO_4 used ml.	Volume of H_2O added ml.	Weight of Zinc g.	Time required min.
1	3	25	none	28
2	3	25	0.5	9
3	3	50	0.3	30
4	3	50	0.5	20
5	5	25	none	19
6	5	25	0.5	12
7	7	25	none	37
8	7	25	0.5	22
9	7	100	none	53
10	7	100	0.5	32

of a considerable number of individual experiments. For example, in Series 6 the times of the individual experiments were as follows: 6, 8, 8, 9, 9, 10, 10, 12, 13, 13, 15, 15, 15, 16, and 19 min. Numerous variables, difficult to control by the method of experiment, such as the temperature of evaporation, the amount of acid lost on evaporation, the sizes of the particles of the salt and the zinc, and the amount of contact of these on stirring, affect the rate of solution. However, since the same procedure would be followed in actual practice it seemed better to design the experiments in this way rather than to attempt a more rigorous control of the variables. Even though there is some overlapping of individual times from one series to another, the average times are distinctly different and clearly significant.

From the results shown in table 2, and from other experiments and observations, the following conclusions may be drawn:

1. Under any given set of conditions the presence of metallic zinc markedly accelerates the rate at which anhydrous ferric sulfate dissolves in dilute sulfuric acid. However, the time required for solution under one set of conditions when no zinc is present may be less than the time required under another set of conditions when zinc is present.
2. There is an optimum range of concentration of sulfuric acid. Not only must the concentration of the acid be high enough to prevent hydrolysis of the

salt on heating but it must be high enough to cause evolution of hydrogen. On the other hand, if the concentration of the acid is too high the rate of solution is retarded either in the presence or in the absence of zinc. Common-ion effect may account for this.

3. Sufficient zinc should be present to be in contact with all the salt. Though fine sub-division of the zinc improves the extent of contact, it cannot be too finely divided or else it will all be dissolved before the salt is dissolved. A large excess of zinc is not only unnecessary, but it may retard the rate of solution because it may reduce the concentration of the acid below the optimum range. However, if the concentration of the acid is initially too high, the addition of a large excess of zinc may have a favorable effect.
4. The efficiency of the process is markedly affected by relatively small changes in conditions. This explains why it is difficult to reproduce results and why such widely different results have been obtained by persons using this procedure for dissolving anhydrous ferric sulfate.

According to the experience of the authors, the following conditions lead to satisfactory results:

1. Add 3.0 ml. of concentrated sulfuric acid for each 0.1 g. of iron that is present.
2. Evaporate on a hot plate at a temperature such that only slight fumes of sulfur trioxide are evolved, and do not continue the evaporation for more than 15 min. after fuming begins.
3. Add 25 ml. of water and 0.5 g. of 20-mesh granulated zinc for each 0.1 g. of iron that is present.
4. Warm the mixture at about 75° C and stir continually in such a way that the salt and zinc remain in contact.

Under these conditions not more than 10 min. should be required for the complete solution of the ferric sulfate.

In solutions prepared in this manner the iron is largely in the ferrous state, and it seems probable that the ferric ions are reduced to ferrous ions at the interface between the solid ferric sulfate and the solution, and that this mechanism accounts for the acceleration of the rate of solution. However, it is also possible that some reduction occurs away from the interface and that this contributes to the shifting of the equilibrium between the ferric and ferrous ions. Other reducing agents, such as stannous sulfate, were tried for their effect on accelerating the rate of solution of anhydrous ferric sulfate in dilute sulfuric acid, but these generally were less effective than zinc and were not of practical utility because of the difficulty of removing the excess of reducing agent before titrating the ferrous iron.

Of all the reagents tried, concentrated hydriodic acid dissolved the isolated ferric sulfate most rapidly. When treated at room temperature with constant boiling hydriodic acid the salt dissolves at once with liberation of iodine but without detectable reduction of the sulfate ion. The following equation indicates the over-all reaction:

$$Fe_2(SO_4)_3 + 2HI \longrightarrow 2FeSO_4 + H_2SO_4 + I_2.$$

When the solution obtained in the cold is heated, or when the salt is treated with the hot acid, the sulfate ion is reduced to yield such products as hydrogen sulfide and sulfur dioxide. Unfortunately, this very rapid method of dissolving anhydrous ferric sulfate cannot conveniently be applied to the usual procedure in which the iron is titrated with permanganate, though it might be applied to a procedure in which the iron is determined indirectly through titration of the iodine liberated in the reaction.

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EFFECTS OF STREPTOMYCIN ON CERTAIN GREEN PLANTS[1, 2]

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While attempting through the use of antibiotics to obtain pure cultures of various algal flagellates and their related colorless forms, Provasoli *et al.* (1948) observed that on exposure to streptomycin (SM), *Euglena gracilis* var. *bacillaris* became colorless. Provided with suitable substrates the bleached organisms could be kept alive in spite of the loss of their photosynthetic apparatus. Complete bleaching resulted from about four days exposure to 100 μg. SM/ml; bleached organisms remained colorless indefinitely and were not inhibited in growth upon subsequent serial transfer in light on SM-free media. Provasoli *et al.* (1951) reported on further experiments with green flagellates and their colorless homologues which led to the hypothesis that SM acts at two different sites in these organisms, one affecting the chloroplast (the *bleaching* site) and the other responsible for killing (the *antibiotic* site). The relative sensitivity of the two sites in a particular species determines whether it will be killed or bleached by exposure to SM. (Although the mode of action of SM is not known, absence of greenness resulting from SM treatment will, for convenience, be referred to as bleaching in this paper.)

Von Euler (1947, a, b) has reported the effect of SM on germinating seeds of barley, rye, and cress. In his experiments SM was observed to have two effects: (1) a general or non-specific growth inhibiting effect resulting in stunting and thickening of the roots and thickening of the coleoptile, and (2) a bleaching effect. Exposure to 0.2 percent SM was sufficient to cause the first and second leaves of the germinating seedlings to be green only at the tips. Higher concentrations resulted in completely white leaves. No observations are reported beyond the seedling stage. Von Euler (1947, a) states that SM does not destroy chlorophyll already formed but that further formation is inhibited.

Ziebur (personal communication) has made preliminary studies of the effect of SM on a number of higher plant species. Both the bleaching and the non-specific growth effects were obtained by a variety of treatments, including treatment of apical buds of nearly mature radish plants. Flower production by shoots treated in this manner made possible some limited observations of progeny resulting from SM-bleached plants. Female sterility and at least partial male sterility were observed. No SM effects were observed in the progeny resulting from the cross, normal female × SM-treated male. The reciprocal cross was not successful.

Other reports of SM bleaching of potentially chlorophyllous tissue include: carrot tumor tissue (De Ropp, 1948), pine seedlings (Bogorad, 1950), wheat and radish (Wright, 1951), and *Chlorella* (Dubé, 1953).

The reports reviewed above raise numerous questions concerning the genetical and physiological mechanism(s) of SM action on plants. Elucidation of these mechanisms has been hampered by the paucity of information regarding SM action on intact higher plants beyond the seedling stage, and by the apparent lack of sexuality in *Euglena*, the organism in which SM action has been most fully

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studied. Because of this situation it seemed desirable to accumulate more information concerning the effects of SM treatments on various plants, and then to examine in some detail those treatments which seemed most likely to lend themselves to clarification of the mode of action of SM. Particularly, an effort has been made to obtain in higher plants the differential effects on bleaching and growth which have been reported for the algal flagellates.

EXPERIMENTAL METHODS

Two experimental methods were employed, (1) seed treatments with species other than those employed by previous workers, and (2) leaf treatment, a modified injection technique applied to more mature plants.

Seed treatment. Attempts were made to maintain seedlings from treated seeds beyond the stage of development reported by previous workers. In an effort to circumvent, at least temporarily, the need for externally supplied metabolites, experiments were conducted with species which are known to be capable of considerable growth at the expense of food stored in the seed. Seeds of pea, bean, and corn were therefore employed.

Typical treatment was as follows: Seed of pea (Burpee's Blue Bantam) were placed in a moist chamber and allowed to begin germination. After 48 hr. they were transferred to filter paper pads in 9 cm. petri dishes. Ten ml. of 1.0 percent SM (Dihydrostreptomycin sulfate, Merck) was introduced into the dish. This volume of solution was sufficient to thoroughly soak the filter paper and to partially submerge the seeds. After 48 hr. in the dishes the seedlings were transferred to quartz sand in waxpaper cartons, four seedlings per carton. The cartons containing the seedlings were placed in the greenhouse or in a darkroom. The plants were watered daily with tap water.

Leaf Treatment. Another means of circumventing the need for exogenous metabolities was to induce bleaching in a part of an otherwise green plant. The bleached portion presumably would be supplied with carbohydrates by the unaffected chlorophyllous tissue of the same plant. The bud treatment experiments of Ziebur, mentioned earlier, are illustrative of this approach.

Another variation of this method was a modified "injection" technique in which bean seedlings were treated by rolling one of the primary leaves slightly and inserting it into a tube containing the SM solution. In most cases treatment was begun at the time of almost complete expansion of the primary leaves, before the bud of the first trifoliate leaf had begun to expand. All leaf treatments were conducted in the greenhouse. Plants were maintained in individual pots and were watered daily with tap water.

RESULTS

Table 1 presents results of a typical experiment in which pea seeds were treated in the manner described in the preceding section. In this experiment control plants, which remained in the moist chamber for 96 hours, were grown in dark and in light under the same conditions as the treated plants. Data reported in the table are from measurements taken after 4 weeks growth, at which time the plants were discarded.

This experiment demonstrated a toxic effect of SM apparently not attributable to its effect on the photosynthetic mechanism. If the effect of SM were only on chlorophyll formation, it would be expected that SM-treated plants which grew in the dark would resemble dark-grown controls. However, treated plants, both in the dark and in the light, were extremely stunted. Roots were short and thick and no lateral roots developed. Stems were extremely short. Although not noted in the table above, the leaves of SM-treated plants, both in the dark and in the light, were less expanded than those of dark-grown controls. Leaves of the dark-grown controls were yellow. Leaves of treated plants, both in the dark and in the light, were white, and hard and waxy to the touch. At the time

that the measurements were made it was noted that the cotyledons of treated plants were still fairly large and firm, indicating that although increase in size of the treated plants had apparently ceased, there was still considerable stored food in the cotyledons. A number of treated seedlings were dying at the conclusion of the experiment, and the cotyledons of a number of them were infected with bacterial colonies. The controls in the light were in flower at the termination of the experiment.

Similar treatment of bean seeds (Burpee's Stringless Green) with SM resulted in bleaching and in the general toxic effects noted above for pea. However, treated plants which grew in the light varied in their appearance. The hypocotyls of all treated plants were extremely stunted. Primary leaves varied in color from pale green to white, and although they unfolded on some plants, they consistently failed to expand. Treated plants failed to develop further than this before dying.

With corn, seed treatments have shown more promise because bleached seedlings seemed to be more resistant to the toxic effect of SM than pea or bean seedlings, although results of seed treatment were extremely irregular. Of the seedlings resulting from the same experiment some were green and apparently normal, others were uniformly pale green, and still others were completely white. A large number had white or partially white primary leaves, but green second leaves. This last group suggests that differences in degree of bleaching result

TABLE 1

Effects of streptomycin on growth of pea plants in light and dark

	1.0% SM light	1.0% SM dark	Control - light	Control dark
Stem length (av.)	1.34 cm.	1.82 cm.	15.94 cm.	30.85 cm.
Root length (av.)	1.50 cm.	1.88 cm.	16.21 cm.	7.60 cm.
Stem color	white	white	green	white
Leaf color	white	white	green	yellow

from differences in the permeability to SM of the seed coat (pericarp) of different seeds.

Bleached corn seedlings, while smaller and slower growing than untreated plants, were relatively less stunted than were the treated pea and bean seedlings. Even completely bleached corn seedlings had fair root development, with considerable branching of the roots. Stunting was roughly proportional to the degree of bleaching, and pale green seedlings grew more than completely white ones, although both died early. The pale green seedlings reached a height of about 8–10 cm. before dying, and the white ones about 3–6 cm. A few of the completely white seedlings were kept alive longer than the rest by moving them from the greenhouse into low-intensity light in the laboratory where they were supplied with carbohydrate by immersion of the tip of one of the leaves in 0.4 M sucrose.

Results of Leaf Treatment Experiments. In an early experiment bean plants were treated in the manner described above with 1.0, 0.5, 0.25, and 0.12 percent SM. Bleaching effects were observable after 9 days. The effects were most striking in the first trifoliate leaf as it expanded, and the intensity of SM effects increased with increase in concentration of SM. (Note: The first trifoliate leaf will hereafter be referred to as TFL-1, the second as TFL-2, etc.)

TFL-1 arises from the main axis at an angle of 90 degrees from the primary leaves. From 0.5 to 0.12 percent SM inclusive, the SM effect was greatest in the portion of TFL-1 which was nearest (*i.e.*, made the smallest angle with) the treated primary leaf. The leaflet nearest the treated primary leaf was most

bleached, the middle leaflet less bleached, and then mostly on the side nearest the treated primary leaf, and the leaflet furthest from the site of treatment was least bleached. The leaflets showed a reduction in amount of expansion which was roughly proportional to the degree of bleaching.

The SM tubes were removed from the leaves after 25 days, at which time the treated leaves were all dead, whereas the primary leaves of controls (immersed in distilled water) were alive.

In this experiment treatment with 1.0 percent SM was lethal. For the lower concentrations, total amount of bleaching in TFL-1 varied with the concentration of SM employed. In the plants treated with 0.5 percent SM the primary leaf opposite the treated leaf developed a mottled necrosis and died prior to the rest of the plant. The plants themselves were dead 29 days after the start of treatment, TFL-2 failing to open. Treatment with 0.25 and 0.12 percent SM permitted limited development of TFL-2. Both bleaching and toxic effects were very slight at the latter concentration. Plants treated with 0.25 percent SM were observed to develop a necrosis in the most severely bleached portion of TFL-1.

By varying SM concentrations and duration of treatments an attempt was made to examine the report of Hutner, *et al.* (1949) that *Euglena* bleaching is a function of total exposure to SM (*i.e.*, duration $\times$ concentration = k). However, it was found that bean plants treated in this manner under greenhouse conditions were, as with seed treatments, not sufficiently sensitive in their reaction to SM treatment to demonstrate an effect from small variations in exposure times and/or SM concentrations. At least 48 hr. treatment was necessary to demonstrate an SM effect at concentrations of 1.0–0.25 percent. Treatment of sufficient duration to cause bleaching generally resulted in the death of the treated leaf.

The use of a wetting agent (0.1 percent Tween-20) in combination with the SM solution appeared to enhance the toxic effect of SM to such an extent that the plants were killed before bleaching could be detected. 1.0 percent SM, even in the absence of the detergent, resulted in the death of the plant without expansion of TFL-1, or if the exposure time was reduced, in no effect at all. Therefore, all subsequent experiments were conducted with SM at concentrations of 0.5 and 0.25 percent.

At these concentrations the minimal exposure necessary to produce bleaching also resulted in toxic effects which severely injured the plants. TFL-2 usually expanded only very slightly. If the plant survived at all, subsequent growth resulted from development of buds in the axils of the cotyledons, primary leaves, and TFL-1 and -2. This would indicate SM injury to the apical growing region resulting in reduction of apical dominance. Even these axillary buds developed only slightly, and the plants usually died without flowering. The axillary shoots, when they developed, had pale green to yellow leaves.

An attempt was made to counteract the toxic effects of SM leaf treatments with 0.2 M sucrose dissolved in the 0.5 percent SM leaf treatment solution. When treated in this manner it was noted that TFL-2 was evenly bleached a pale yellow to white. The characteristic pattern of bleaching (*i.e.*, bleaching inversely proportional to the distance of the tissue from the site of treatment) which developed in the plants treated with SM alone, was absent. In the presence of added sucrose the compound leaves apparently were completely lacking in chlorophyll, and since both primary leaves were usually lost after treatment the plants were virtually devoid of chlorophyll and thus prevented from further growth. The lives of a few such plants were prolonged by immersing the center leaflet of TFL-1 in 0.4 M sucrose after the SM treatment. This treatment resulted in prolonging the lives of some of these plants by about a week beyond that of SM treated controls. TFL-1 of plants treated with SM and sucrose expanded uniformly to about one-fourth to one-third the width of TFL-1 on untreated plants.

A few attempts were made to induce bleaching in more mature plants in the

hope that they would be able to reach the flowering stage in spite of toxic effects. Although such efforts were not successful it was noted that when TFL-1 of a plant having three TFL's was treated, TFL-4 was white when it expanded, expansion being about one-third of normal. No appreciable effects were discernible in the intervening leaves.

In these experiments the bleaching effect was manifested only in immature leaves. Leaves which were already expanded and green could not be made to bleach by SM treatment. Treated primary leaves died without bleaching, and the primary leaves opposite the treated ones, although often exhibiting a toxic effect in the form of a spotty brown necrosis, did not develop the characteristic SM-induced bleaching.

Toxic effects resulting from leaf treatments were at least as profound as from treatments of germinating seeds. In many bean plants which were leaf-treated with 0.12 and 0.25 percent SM the toxic effect persisted much longer than did the bleaching. Bleaching was most evident in TFL-1 and -2. The most marked effects on leaves which developed later, whether they were derived directly from the apical meristem or from axillary buds, were toxic effects such as reduced size, puckering, and abnormalities in the shape of the leaflet blades.

DISCUSSION

The experiments described above extend the number of species of green plants known to be "bleached" by exposure to SM. In addition, a new method for treatment of plants with SM (*i.e.*, leaf treatment) is described.

The present investigations have failed to reveal the marked difference in sensitivity to the bleaching and toxic actions of SM reported by Provasoli and coworkers for *Euglena* (and suggested for other algal flagellates). Rather, in the present experiments bleaching was always accompanied by toxic effects, and both bleaching and toxicity were intensified as the SM concentration was increased within a certain range (0.12–0.50 percent). In pea and bean the toxic effect of SM is evidenced by suppression of lateral root formation, stunting and thickening of roots and stem, and inhibition of leaf expansion.

Neither the seed treatments nor the leaf treatments described here have yielded highly uniform and reproducible results. Under greenhouse conditions it was found that fairly uniform results were often obtainable with plants within a single experimental series, but that series run at different times often differed widely in reaction to the same treatment. For this reason and also because of the apparently lower sensitivity to SM of higher plants as compared to *Euglena*, it has not been possible to confirm the claim made for the latter organism that bleaching is a function of total exposure.

Attempts to reduce toxicity without reducing bleaching, by shortening the exposure time or by reducing the concentration of SM have not been successful. However, corn appears to be less susceptible to the toxic action than the other species tested, and provision of carbohydrate by immersion of leaf tips in sucrose may provide a means of prolonging the life of bleached plants of corn and other species.

The wide variety of plants which are profoundly affected by exposure to low concentrations of SM is of considerable interest, particularly with regard to the mechanism(s) of action of this naturally occurring compound. The marked general toxic effects of low concentrations of SM are worthy of further attention in their own right. These effects range from the well known bactericidal action on numerous pathogens to the stunting effects reported here and elsewhere for higher plants. The fundamental nature of these effects is indicated by the report of Tanaka and Satô (1952). They conclude from observations of the effect of SM on mitosis in onion root tips that SM is a mutagenic agent.

The bleaching effect as reported from studies with *Euglena*, which suggests

that SM induces a "heritable" somatic variation, is particularly interesting. It may be assumed that studies on the transmission of the bleaching effect to sexual progeny, both in protists and in flowering plants, will shed light on the mechanism of SM action and on plastid inheritance. But in order to examine the effect of SM on sexual progeny of bleached plants it will first be necessary to reduce toxicity without reducing bleaching, or else to find a plant which is insensitive to the toxic action of SM while susceptible to bleaching.

SUMMARY

Germinating seeds and portions of plants at later stages of development have been treated by exposure to stretomycin. Within a certain range of low concentrations of streptomycin (0.12–1.0 percent) this treatment results in absence of greenness in potentially chlorophyllous tissue, and in toxic effects such as stunting of stems, roots, and leaves.

The plants investigated were pea, bean, and corn. Seed treatment and leaf treatment were employed as means for exposing plants to streptomycin. Seed treatment consisted of placing germinating seeds in streptomycin for varying lengths of time, followed by non-aseptic cultivation in quartz sand. Leaf treatment, attempted only with bean, consisted of immersing one of the primary leaves of an intact plant in streptomycin solution prior to expansion of the first trifoliate leaf.

The toxic effects of streptomycin on these plants may be independent of the bleaching effect. However, contrary to reports regarding streptomycin treatment of some algal flagellates, it was not possible to obtain bleaching without toxic effects, although less marked toxic effects were noted in corn than in the other species tested.

In leaf treatments of immature bean plants streptomycin effects are manifested primarily in the first and second trifoliate leaves when these expand. Both bleaching and toxic effects are greatest in the portions of the trifoliate leaves which are nearest to the treated primary leaf. The use of sucrose in combination with streptomycin in these experiments results in a more even distribution of the bleaching effect in the trifoliate leaves.

In a few experiments the lives of plants subjected to lethal treatments with streptomycin were prolonged by supplying sucrose through the leaves.

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STUDIES OF THE GENUS EMPOASCA. XIII. SEVEN NEW SPECIES OF MEXICAN EMPOASCA

HOMOPTERA: CICADELLIDAE

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Among the previous species of *Empoasca* which have been described from Mexican specimens, five at least have elytra which appear mottled in color, having paler areolar spots. Some are heavily marked, others are more delicate in color. This group includes *E. acantha*, *adexa*, *ingena*, *obstipa* and *serrula*. In the following pages seven species are described all of which are mottled in color and are apparently rather closely related to the five species named above.

The name *Empoasca atinca* is proposed for the species described as *E. jacinta* DeLong in the Ohio Journal of Science, page 168, vol. 52, May, 1952. The name is preoccupied by *E. jacinta* DeLong and Davidson, 1936.

The types of all species are in the DeLong collection.

Empoasca metana n. sp.

Resembling *adexa* in general appearance and coloration but with distinct male genitalia. Length 3.5 mm.

Crown produced, blunt at apex, at least one-third wider between eyes at base than median length.

Color pale brown with a median pale stripe from base of crown across apex and extending across face to clypeus. Pronotum with a median pale longitudinal line and a pale spot behind each eye on anterior margin. Scutellum with apex pale. Elytra with anterior portion hyaline, clavus and corium reddish brown with four staggered pale areolar spots, two on clavus and two on corium. A series of pale connected spots angularly sloping across elytra just posterior to clavus. Apices smoky, veins pale margined.

Genitalia: Female seventh sternite broadly and slightly roundedly produced on posterior margin. Male styles narrowed with sharp pointed apices which are curved outwardly. The lateral pygofer processes are slender, almost straight, tapered to pointed apices which are slightly curved inwardly at their apices. The aedeagus with a process arising anteriorly a little before apex which extends anteriorly then dorsally to about the length of aedeagus. The pygofer hook is rather broad at base. The apical third is narrowed to a long pointed apex which curves ventrally and slightly anteriorly.

Holotype male, allotype female and male paratypes collected at Mexico City, D. F.,September 13, 1939, by DeLong. Male paratypes collected at Puebla, Pue., Mexico, October 18, 1941, by DeLong, Good, Plummer and Caldwell.

Empoasca aracoma n. sp.

Resembling *acantha* in general appearance but with distinct male genital structures. Length 3 mm.

Crown rather broad and blunt on anterior margin about one-fourth wider between eyes at base than median length.

Color pale brownish green. Face with a distinct white cross between eyes. Margin of crown pale, connecting with a median pale longitudinal line and an elongate pale spot next each eye. Pronotum with a pale median longitudinal line, posterior margin pale and with a large pale spot just back of each eye. Basal angles, median line and apical half of scutellum pale. Elytra with veins broadly pale margined with dull green or smoky. The greatest amount of pigment is along the veins posterior to the clavus. The palest coloration is on the clavus and corium.

Genitalia: Female seventh sternite with posterior margin appearing truncate. Male styles gradually narrowed on apical half to outwardly curved pointed apices. The lateral pygofer

processes are long, rather narrow with outwardly curved narrow pointed apices. The aedeagus is erect with a process arising anteriorly just before the apex which extends anteriorly then dorsally. The pygofer hook is sickle shaped with the apex downward. The middle portion is thickened, the apical portion is narrow, elongate, sharp pointed and extends ventrally.

Holotype male, allotype female and male paratype collected at Guadalajara Jal, Mexico, October 3, 1941 (Km 645) by DeLong, Plummer, Caldwell and Good.

Empoasca tolana n. sp.

Resembling *serrula* in general form and appearance with different coloration and male genitalia. Length 3 mm.

Crown short, bluntly rounded, almost twice as wide between eyes at base as median length.

Color of face rusty with a median pale longitudinal line. Crown dull greenish tinged with red, with a median pale stripe and a minute pale spot on each side near base. Pronotum yellowish brown with three pale anterior spots, one at middle and one just back of each eye. Scutellum with a pale median longitudinal stripe and a pale spot at apex. Elytra whitish hyaline, costal portion unmarked, yellowish areas tinged with red on clavus and corium. Veins of apical portion white, bordered with smoky.

Genitalia: Female seventh sternite slightly produced and broadly rounded on the posterior margin. Male narrowed on apical half with the apices pointed and curving outwardly. The lateral pygofer processes slope inwardly and are concave on inner margin just before pointed apex which appears turned inwardly. The aedeagus has a short thick anterior process which arises just before the apex. The pygofer hook is broad, rather short and pointed on the anterior ventral margin.

Holotype male, allotype female and male and female paratypes collected at Coscomatepec, Veracruz, Mexico, September 30, 1924, by Dr. A. Dampf. Female paratypes collected on the Acapulco Rd., Mexico, November 22, 1938, by Dr. J. S. Caldwell.

Empoasca mosella n. sp.

Resembling *adexa* in general form and coloration but with different male genitalia. Length 3 mm.

Crown blunt, broadly curved anteriorly, about one-fourth wider between eyes at base than median length.

Color dark reddish brown, a median longitudinal pale line on face which extends onto crown. A white spot either side on margin of crown and a larger spot next each eye at base. Pronotum with a median paler longitudinal stripe, a small pale spot just back of each eye and one on each humeral angle. Scutellum with a pale spot on each side of apex. Elytra reddish brown with five round areolar spots on basal two thirds. A series of three pale spots on each side arranged transversely at apex of clavus. Apex smoky with pale veins.

Genitalia: Female seventh sternite rather strongly produced and convexly rounded on posterior margin. Male styles narrowed on apical half to form sharp pointed apices which curve outwardly. The lateral pygofer processes are long, narrow and with narrow pointed apices. The aedeagus forms an anterior process at about its middle which extends forward for some distance and this gives rise to a dorsally produced process near its apex which is slender and extends parallel to the aedeagus shaft and to about its length. The pygofer hook is thickened at base, is sickle shaped with the apical half narrow and with a sharp pointed apex which extends ventrally and anteriorly.

Holotype male and allotype female collected at Carapan, Mich. (Km. 432) Mexico, October 2, 1941; male paratypes collected at Zimipan Hgo (K. 223), October 30, 1941, and September 26, 1941, by DeLong, Plummer, Caldwell and Good.

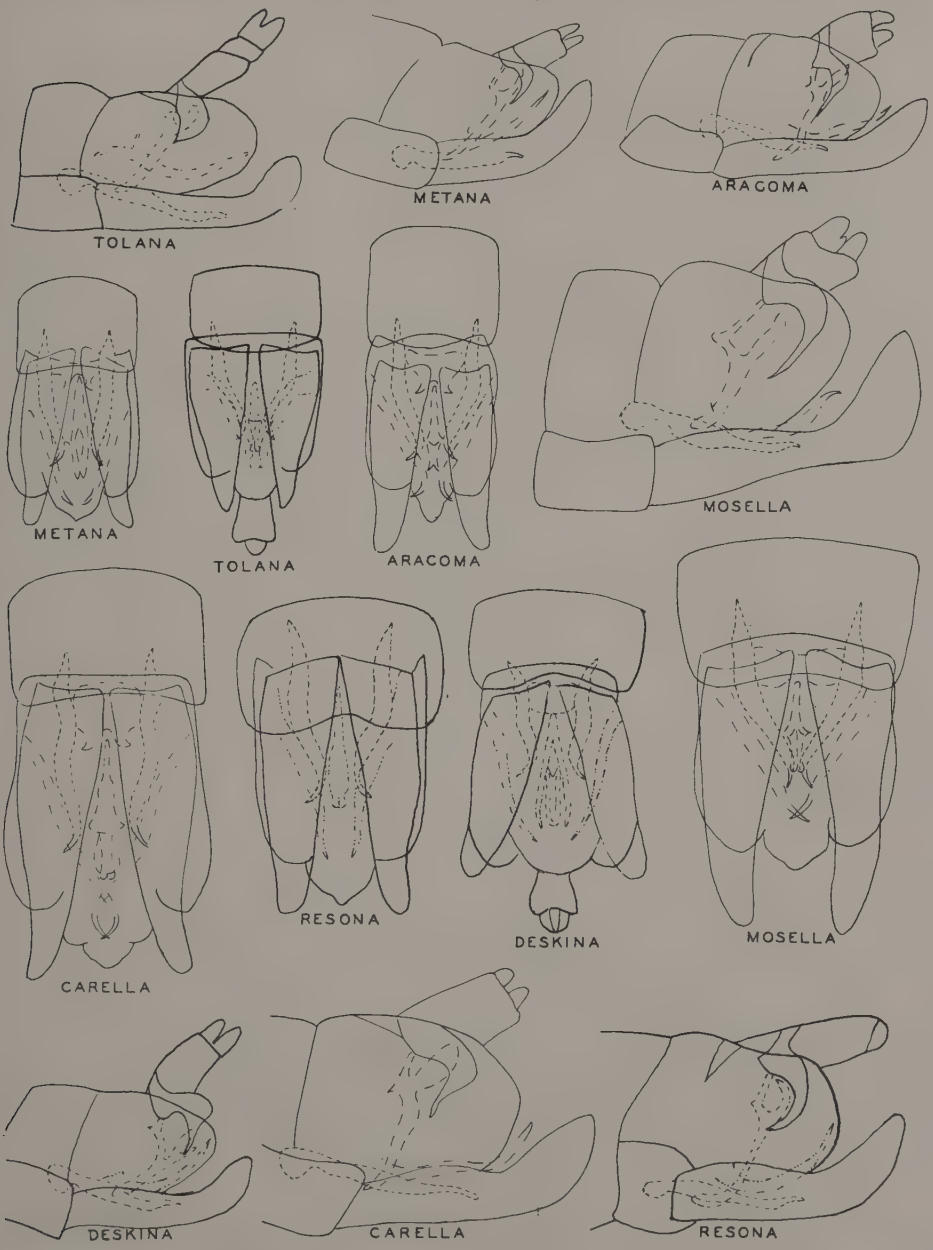
Empoasca resona n. sp.

Resembling *acantha* but with different coloration and male genital structures. Length 3.5 mm.

Crown produced, blunt, rounded, almost one third wider between eyes at base than median length.

Color of face sordid with a pale median longitudinal stripe. Crown embrowned at apex with a median pale stripe and a pale spot either side on margin. Portion next eyes pale. Pro-

notum brown with a pale spot just back of each eye and pale humeral angles. Scutellum brown, a pale spot each side of apex. Elytra with a yellow costal margin. The clavus is brownish with two white areolar spots. The portion of corium along clavus is reddish with two pale areolar spots. Apex smoky, veins pale. A series of pale spots transversely arranged at apex of clavus.



Lateral and ventral views of the apical portion of the abdomens of the males of species of Empoasca as labeled.

Genitalia: Male style narrowed and curved outwardly at pointed apex. The lateral pygofer processes are long, the apical third is quite slender with long attenuated pointed apices. The aedeagus is curved anteriorly at apex. The anterior process arises near the apex and extends anteriorly then dorsally and is as long as the aedeagus shaft. The pygofer hook is rather long, thickened near base then gradually narrowed to a pointed apex which extends ventrally and is curved anteriorly.

Holotype male collected at Carapan, Mich., Mexico (Km. 432), October 2, 1941, by DeLong, Plummer, Caldwell and Good.

Empoasca corella n. sp.

Resembling *adexa* in coloration and general appearance but with distinct genital structures. Length 3.5 mm.

Crown bluntly produced about one-fourth wider between eyes than median length. Elytra much longer than abdomen.

Color crown and face rather dark brown, a median pale stripe on face from margin of crown to clypeus. Crown with a median longitudinal pale line, a large pale spot next eye on base at either side and a small pale spot on margin between apex and eye. Pronotum dark brown with three pale spots along the anterior margin, one at middle and one behind each eye. Scutellum with basal angles and apical angle pale. The elytra are dark brown with three white areolar spots on each side along commissure. The posterior spot is just outside the clavus at its apex. The claval vein is reddish. The apical veins are pale. The median costal margin is pale.

Genitalia: Female ventral sternite broadly roundly produced on posterior margin. Male styles elongate, slender, with outwardly bent pointed apices. Lateral processes long, quite slender, apices pointed, curved outwardly. Aedeagus indented on posterior margin at about two-thirds its length forming a rounded, elongate apical lobe and an anterior process with a ventral pointed outer margin and a dorsal narrow produced finger like process. The pygofer spine is thick at base, sloping caudally then bent anteriorly forming a narrow pointed apex which extends ventrally.

Holotype male, allotype female and male and female paratypes collected at Carapan, Mich., Mexico (Km. 432), D. F., Mexico, October 2, 1941; male and female paratypes from Tres Cumbres (K. 52), D. F., Mexico, October 21, 1941; Cordoba Veracruz, Mexico, October 8, 1941. Collected by Caldwell, Good, Plummer and DeLong. Paratypes from Taxco, Gro., Mexico, Sept. 9, 1939, collected by Plummer and DeLong.

Empoasca deskina n. sp.

Resembling *adexa* in general appearance and coloration but with distinct male genitalia. Length 3.5 mm.

Crown rather broad, rounded in front, almost twice as wide between eyes at base as median length.

Color rather dark brown, a pale line from margin of crown to clypeus which is black. Crown with a pale spot next each eye at base and a small pale spot on each side of anterior margin. Pronotum brown with a median pale spot on anterior margin and one just back of each eye. Scutellum pale at apex. Elytra brownish with two pale areolar spots on clavus, one basal, anterior to clavus, one on corium and a series across elytra just posterior to clavus. Apices smoky with pale veins.

Genitalia: Female seventh sternite slightly produced and broadly rounded on posterior margin, male styles long and slender, apices pointed and turned outwardly. Lateral processes long, tapered to slender apices. Aedeagus branched just beyond middle forming a long slender anterior process which extends dorsally and anteriorly and a posterior portion which is straight, slender and with a posterior slender spur just before its apex. The pygofer spine is broad with anterior ventral excavations and a narrow pointed elongate apex which extends ventrally and anteriorly.

Holotype male from Cuernavaca, Mor., Mexico (K-57), collected October 21, 1941. Female allotype and paratype, female from Jacala Hgo., September 26, 1941. Male and female paratypes from Mexico, D. F. (Km. 43 S.), September 5, 1939; Orizaba, Veracruz (K-280), October 17, 1941, by DeLong, Plummer, Good, and Caldwell. Paratypes from Mexico, D. F., September 19, 1932, by Dr. A. Dampf.

ABNORMAL DEVELOPMENT IN *RANA PIPIENS* INDUCED BY COLCHICINE TREATMENT AT LATE GASTRULATION

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The narcotic alkaloid, colchicine, has been used extensively for a number of years in the experimental study of polyploidy in plants. It has been used to a less extent on animal tissue.

Among vertebrates, Waterman (1940) has found that colchicine has general retardative and inhibitory effects on the fish embryo, *Oryzias latipes*. This was indicated by developmental disturbances in the brain and eyes, the circulatory system, and in modifications of the tail.

Paff (1939) noted general retarded growth and anomalies of the nervous system of chick embryos after colchicine treatment. In addition, he produced evidence to show that colchicine may have an acceleratory effect as well as an inhibitory effect on mitosis.

Keppel and Dawson (1939) treated very early stages of *Rana pipiens* with colchicine in dilute concentrations over relatively long exposure periods. The results showed that colchicine induces a meroblastic type of cleavage and disturbs gastrulation. There was some indication of polyploidy. Rarely were embryos viable beyond the neural fold stage.

The eggs of *Rana pipiens* were also used in the present investigation. In contrast to the experiments of Keppel and Dawson, however, exposure to colchicine was at a later stage, at much higher concentrations, and for relatively short periods of time. Gastrulation was the stage chosen for treatment since the period up to, and perhaps through, this stage seems to be the most critical and because the effect of colchicine on organogenesis could be observed readily.

MATERIALS AND METHODS

The eggs for these experiments were obtained during the spring by injections of crushed pituitary glands and were fertilized artificially according to Rugh (1941).

All eggs were fertilized at room temperature, cut with dissecting scissors into groups of five, and then kept in finger bowls in a large, well insulated constant temperature cabinet at 18.0°C ± 0.3°C. The cabinet contained a light source that switched on and off automatically.

After fertilized eggs were placed in the constant temperature cabinet, development was allowed to continue normally for 42-43 hours (except one batch, series 14, in which development continued for 46 hours); i.e., embryos were permitted to develop until they had reached a stage in gastrulation between Shumway's (1940) stages 11 and 12 (mid- to late gastrula). Gastrulae then were placed in their respective colchicine concentrations (cooled to 18°C before use) in fresh greenhouse tank water. Control groups were placed in fresh greenhouse tank water. In general, embryos were fixed in Smith's fluid (Rugh, 1941) four days after treatment; *i. e.*, when the controls were at approximately Shumway stage 20 (hatching stage). Some of the embryos, however, were fixed in Smith's fluid twenty-four hours after treatment. Each series represents 140 eggs from a single female; 14 females were used. Treatment used is summarized in table 1.

All preserved series were divided into three groups on the basis of length of

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exposure to colchicine and the form of colchicine used (table 2); subdivisions for the various concentrations were retained in each group.

Puckett's dioxan technique (1937) was used in running up embryos for embedding in 56-58°C. paraffin. Embryos were sectioned at ten micra and stained with Delafield's hematoxylin and eosin.

RESULTS

A summary of the abnormalities and mortality obtained at the different colchicine concentrations for one- and four-hour exposure periods is given in table 2.

Observations of Living Material

For most of the series, colchicine treatment was begun about 4:00 P.M. By 7:30 A.M. of the next day, the retarding effect of colchicine was observable already in that all the embryos in the higher concentrations were still in the late gastrula stage. Usually after about twelve hours of colchicine treatment (longer when the crystalline form was used) a few scattered clumps of cells were observed in the dorsal lip region of some of the embryos in the higher concentrations. During the remaining part of the four days in which these embryos were allowed to develop,

TABLE 1

Treatment of Rana pipiens *at Late Gastrulation*

Series	Form of colchicine used	Colchicine concentrations	Exposure period	Fixation
1–6	Fresh amorphous powder	0.1%, 0.01%, 0.001% 0.0001%, 0.00001%, 0.000001%, plus control	1 hour	½ fixed 24 hours after treatment; ½ fixed 4 days after treatment.
7–10	Old crystals (kept in laboratory at least 2 years)*	0.4%, 0.2%, 0.1%, 0.05%, 0.01%, 0.001%, plus control	1 hour	Fixed 4 days after treatment
11–12	Fresh amorphous powder	0.4%, 0.2%, 0.1%, 0.05%, 0.1%, 0.001%, plus control	4 hours	Most fixed 4 days after treatment
13–14	Fresh amorphous powder	1.0%, 0.7%, 0.4%, 0.2%, 0.1%, 0.05%, plus control	1 hour	Most fixed 4 days after treatment

*Fresh powder unavailable for a short time.

these small light-colored clumps of necrotic cells increased in number, appearing to increase from the dorsal lip region forward. In the higher concentrations these sloughed off clumps of cells eventually formed a thick mass of cells along the neural groove. In these cases the closing of the neural folds was inhibited. Some of these clumps of necrotic cells appeared to be kept in constant motion within the inner jelly capsule by the ciliary movements of the embryo. These accumulations of sloughed-off cells were progressively less conspicuous in the lower concentrations.

Many of the experimental animals retained their yolk plugs much longer than did the controls. This was especially true of embryos in the higher concentrations, those in 1.0% colchicine retaining their yolk plugs the longest. In Series 14, 1.0% concentration, all animals fixed had persistent yolk plugs. A hatched embryo with a persistent yolk plug sometimes was observed moving around with yolk plug protruding; in others, exuding yolk was strewn around in the medium. This latter effect may be duplicated by stimulating the embryos with an instrument, after which muscular contraction "caused the yolk to exude," as Dawson (1938) aptly put it , "as a solid core resembling paste leaving a tube."

Most of the weakened embryos in the higher concentrations had failed to hatch

at fixing age, while only a few of the controls were unhatched when fixed. This was probably a natural result of the inhibitory effect of colchicine. Waterman (1940) also mentions the failure of weakened fish embryos to hatch after colchicine treatment.

Mortality of treated animals (table 2) was mainly in three batches, and apparently is correlated with concentration and length of exposure.

EXTERNAL OBSERVATIONS

The treated animals, in general, seem to fit into a graded series, the effect of the

TABLE 2

Abnormalities and Mortality of Treated Embryos
(Based on external observations)

	Conc. %	Total Embryos Treated	Mortality of Treated Embryos	Ratio Abnormals to Total Sample Treated	Abnormals %
	1.0	40	23	40/40	100.0
	0.7	40	22	40/40	100.0
Group I	0.4	39	22	39/39	100.0
	0.2	40	4 plus 1?	40/40	100.0
Series	0.1	94	1 plus 5?	94/94	100.0
1–6, 13,	0.05	40	1	22/40	55.0*
and 14	0.01	59	0	4/59	6.8
	0.001	60	0	1/60	1.7
Powder	0.0001	58	0	5/58	8.6*
1 hour	0.00001	59	0	3/59	5.1*
	0.000001	60	0	0/60	0.0
	Control	99	0	4/99	4.0*
Group II	0.4	79	2	79/79	100.0
	0.2	80	1	77/80	96.3*
Series	0.1	76	0	21/76	27.6*
7–10	0.05	79	0	1/79	1.3
	0.01	80	0	4/80	5.0*
Crystals	0.001	80	0	5/80	6.3*
1 hour	Control	78	0	6/78	7.7*
Group III	0.4	40	30 plus 7?	40/40	100.0
	0.2	40	18 plus 3?	40/40	100.0
Series	0.1	40	11 plus 7?	40/40	100.0
11 and 12	0.05	40	5 plus 2?	40/40	100.0
	0.01	39	0	0/39	0.0
Powder	0.001	40	0	0/40	0.0
4 hours	Control	39	0	2/39	5.1*
Total	Controls	216	0	12/216	5.6*

‡Embryos treated minus those killed accidentally as a result of handling.
*Many of the embryos represented by these percentages were only slightly abnormal in appearance.

colchicine treatment increasing with increasing concentrations. There is a general inhibitory effect resulting in stunted growth. One of the most marked effects is a gradual reduction in head size until the most antero-dorsal part of the embryo is on a line with the suckers instead of protruding beyond them as in the controls. Varying degrees of microcephaly have also been noted by Hoadley (1938) when embryos were kept at supramaximal temperatures, and by Briggs (1941) when embryos were permitted to develop following delayed fertilization. Other marked effects noted in the present investigation are total inhibition, partial retardation,

TABLE 3

*Summary of Histological Observations**

	COLCHICINE CONCENTRATIONS						
	1.0%	0.7%	0.4%	0.2%	0.1%	0.05%	0.01–0.00001% and control
Suckers	Almost normal development	Almost normal development	Normal	Normal	Normal	Normal	Normal
Olfactory pits	Absent	Absent	Absent	Absent	Absent	Absent	Normal
Eyes	Absent	Absent	Absent	Absent	Optic stalk and optic vesicles present; no lens	Optic stalk and cup show infiltration; lens present	Normal: optic stalk, vesicles, and lens
Otic vesicles	Absent	Absent	Absent	Absent	Differentiated but retarded	Normally differentiated; abnormal in position	Normal
Brain	Absent	Absent	Absent	Solid cord of cells surrounded by cavity	Differentiated but retarded	Larger than in 0.1% but still retarded	Normal: cavities free of infiltrated cells
Nerve Cord	Solid mass of indifferent cells	Solid mass of indifferent cells	Solid, some cellular differentiation	Solid and differentiated	Tubular; contains infiltrated cells	Tubular; normal differentiation; heavy infiltration	Normal: tubular, and canal free of cells
Hypophysis	Absent	Absent	Absent	Absent	Anlage present	Normal?	Normal
External gills	Absent	Absent	Absent	Absent	Anlage present	Arteries present; retarded	Normal, containing afferent arteries
Thyroid	Absent	Absent	Absent	Absent	Anlage present	Normal	Normal
Gut	Blocked with yolk; undifferentiated lining	Blocked with yolk; undifferentiated lining	Blocked with yolk; lining regular in outline	Mid-gut and pharynx differentiated (infiltrated)	Differentiated and free of foreign cells	Normal	Normal
Liver	Absent	Absent	Absent	Absent	Present?	Present?	Normal
Notochord	Filled with cells containing yolk and pigment granules	Filled with cells containing yolk and pigment granules	Fewer yolk-containing cells	Yolk present	Almost free of yolk	Normal	Normal: vacuolated and free of foreign cells
Heart	Absent	Absent	Absent	Absent?	Primordia?	Atrium and ventricle present; abnormally small	Normal: atrium and ventricle
Pericardial cavity	Absent	Absent	Large somewhat irregular cavity	Present; dorsal mesocardium connected to yolk	Abnormally large	Abnormally large; ventro-posterior yolk mass	Normal
Somites	Undifferentiated mass	Undifferentiated mass	Slightly differentiated	Indistinct	Incompletely differentiated	Differentiated	Normal
Coelom	Absent	Absent	Indicated by right and left cavities	Irregularly formed	Differentiated	Normal?	Normal
Pronephric tubules	Absent	Absent	Absent	One tubule (left)	Present	Normal?	Normal

**Rana pipiens* embryos treated with colchicine solutions of given concentrations for one hour, compared with untreated (normal) embryos (Shumway stage 20–21); incubation period, 140 hours; temperature, 18° C.

or malformation of tails; an increasing failure to close the blastopore; mild oedema; reduction or complete inhibition of external gills; and a dorsal flexion of the tail giving a "swayback" appearance to embryos in the intermediate concentrations. Waterman (1940) noted a number of these effects on fish embryos.

Effects which do not seem to be progressive but rather occur at random throughout the concentrations producing abnormals are the following: (1) marked oedema giving the embryos a bloated appearance, and (2) occurrence of smooth-surfaced, ectodermal, irregularly placed enlargements, the latter noted mainly in the dorsal region. The latter appear to be similar to the epidermal "blisters" noted by Mills (1939) after treating hatching tadpoles with colchicine. In the present experiments, an occasional extremely bloated embryo was permitted to develop beyond the four-day period that was allotted the others after colchicine treatment. Inflation increased in these animals until they died four or five days later.

It was found desirable to separate the series treated with fresh amorphous powder from those treated with the somewhat aged crystals (used for a short time when fresh powder was unavailable), and those treated for four hours from those treated for one hour. The powder used appeared to be more toxic than the crystals and the four-hour treatment more effective than the one-hour treatment (table 2).

Histological Observations

Histological studies are based on serial sections chiefly of the embryos of group I, one-hour powder treatment. These results are summarized in table 3. Because of apparent batch susceptibility, care was taken to section examples of the same batch wherever feasible. The embryos treated with a 1.0% colchicine solution and those treated with a 0.7% solution are very similar. The extreme retardation of these embryos which have persistent blastopores may be noted in table 3. The ventral yolk mass is reduced caudal to the head region, as indicated by an irregular cavity containing scattered clumps of yolk. Yolk is found in all the internal structures mentioned, as are scattered pigment granules. What appear to be abnormally large nuclei are present in the notochord and somites.

An embryo treated with a 0.4% concentration is, in gross appearance, similar to those in the two higher concentrations. Internally, however, it is not markedly inhibited (table 3). The pericardial cavity appears as a large, somewhat irregular space, divided dorso-ventrally into two parts by a mass of cells containing yolk. Small coelomic cavities are indicated by two spaces (right and left) located laterally and ventral to the notochord. The spinal cord region shows slight differentiation as compared with the control. The notochord contains fewer cells infiltrated with yolk granules so that it appears more vacuolated. The somites show some differentiation, and the gut lining is regular in outline, though the gut cavity still is blocked with yolk.

In a sectioned embryo which had been treated with the 0.2% solution (table 3), a solid cord of cells enclosed by a cavity appears in the prospective brain region. The nerve cord is still solid but differentiated. The mid-gut has a differentiated cellular lining. Indistinct somites are present, as is an irregularly formed coelom and a left pronephric tubule. The pharynx is differentiated but contains a few cells infiltrated with yolk. The pericardial cavity and what appears to be the dorsal mesocardium are present, but the latter is connected ventrally to a mass of yolk lying in the floor of the pericardial cavity.

The sections of an embryo treated with a 0.1% concentration (table 3) show a differentiated but retarded pharynx and brain. The primordia of the thyroid and hypophysis are present. The optic stalks and optic vesicles are present but the lens did not form. Otic vesicles containing a few cells infiltrated with yolk differentiated but are retarded in development. A nerve cord differentiated with a neurocoel but the latter contains a loose mass of cells. External gill primordia are present as evidenced by a slight protrusion on both sides of the head. Also

present are an abnormally large pericardial cavity, incompletely differentiated somites, a gut cavity free of foreign cells, pronephric tubules, a differentiated coelom and a vacuolated notochord practically free of cells containing yolk. The pericardial cavity merges caudally into an irregular cavity beneath the gut. The sections show a loose mass of infiltrated cells dorsal to the neural tube, caudal to the pericardial region.

An embryo treated with 0.05% concentration of colchicine has all the structures indicated in table 3 except the olfactory pits. The brain is larger than in the 0.1% concentration but still retarded, and the otic vesicles are normal as to differentiation but abnormally posterior in position. The external gills contain afferent branchial arteries but are somewhat retarded in development as compared with the control. The pericardial cavity is abnormally large. The tubular nerve cord is differentiated but is infiltrated heavily with cells, as are the cavities of the optic stalk and the optic cup. The heart is abnormally small and a mass of yolk is present in the posterior portion of the floor of the pericardial cavity.

All sectioned embryos of group I treated with more dilute concentrations than those described above appear normal.

Embryos of group III that were sectioned serially indicate, as did those of group I and group II, that increasing the concentration increased the toxicity of the drug. Sections demonstrate, however, that the toxic effects in group II (crystalline form) are not as great as those in group I where powdered colchicine was used; and the toxic effects in group I (one-hour treatment) are not as great as those in group III (four-hour treatment) for any given concentration.

DISCUSSION

The present investigation deals with the effects of colchicine on one stage of development in the frog, namely, the yolk plug stage. Since this research was done, Colombo (1944, 1947) and Samartino and Rugh (1946) have reported the effects of colchicine on various stages in frog development. The concentrations that these authors used, however, did not include concentrations as high as the present writer used. Colombo used longer exposure periods (ranging from one to twenty-five days) than those used in the present investigation. Samartino and Rugh did not indicate the length of exposure to colchicine. The present paper includes the effects of relatively high concentrations (up to 1.0%) acting for short exposure periods (one hour and four hours). The results demonstrate that gastrulae of *Rana pipiens* are able to tolerate colchicine concentrations up to 0.01% for short periods without teratological effects, and may live several days following exposure to chochicine. At 0.05% concentration, the four-hour powder treatment was more effective than the one-hour powder treatment in producing abnormalities.

The toxicity of colchicine has been shown to be correlated to some degree with temperature. Fühner, and Fühner and Wagner (Fühner and Breipohl, 1933) observed that colchicine is far more toxic at warm temperatures than at cold. The present work tends to confirm this observation since in preliminary experiments a high percentage of abnormalities was obtained in concentrations of 0.01% and 0.001% when yolk plug stages were treated for one hour at room temperature (22-24°C), while at a temperature of 18°C no significant percentage of abnormalities was obtained. It was, therefore, necessary to resort to higher concentrations in order to obtain a graded series of abnormalities at 18°C, a temperature convenient for comparison with Shumway's stages for the development of *Rana pipiens* (1940). Colombo (1947) states that *Rana agilis* appears to be much less susceptible to the action of colchicine than is *Rana esculenta* which he used in his first series of experiments (1944). He attributes this, in part, to a difference in rate of development between the two species and, in part, to the difference in temperature at which the two series of experiments were run (*Rana agilis* at 7-11°C and *Rana esculenta* at 17-23°C). He states that the diffusibility of colchicine into the cytoplasm would probably be faster at higher temperatures and vice versa. His results, therefore,

tend to confirm the observations presented here. It should be pointed out, however, that raising the temperature may induce teratological effects (Hoadley, 1938) by abnormally increasing the rate of development and thus may be producing an indirect effect.

The most marked effect of colchicine noted on living embryos, besides general retardation, was the sloughing of cells beginning around the dorsal lip and later extending more anteriorly in the region of the neural groove. The ciliary motion of the developing embryos probably freed these clumps and then set them moving in every direction. As more and more of these clumps of cells became free and finally congregated along the wall of the inner capsule, it was difficult to determine whether an embryo was alive unless it was actually observed moving within its capsule. This effect was due apparently to the death of the sloughed cells which congregated along the inner wall, causing the capsule to appear whitish and opalescent. Paff (1939) *et al* noted a sloughing of the ectoderm in the chick embryo after colchicine treatment. Solberg (1938) noted the extrusion of injured cells from tissues following x-ray treatment. The fact that the sloughing began at the dorsal lip is in accord with his observations that during gastrulation the dorsal lip and the invaginating tissue are the most sensitive parts of the fish embryo.

The persistent yolk plugs obtained after colchicine treatment have also been noted after dinitrophenol treatment, of *Rana pipiens* (Dawson, 1938) and of fish embryos (Waterman, 1939). Persistent yolk plugs and delayed closure of the blastopore also have been obtained by keeping developing eggs at subminimal and supramaximal temperatures (Hoadley, 1938; Atlas, 1935). Hoadley (1938) and Dawson (1938) mention loss of yolk and atypical closure of the blastopore. Waterman (1940) mentions the failure of weakened embryos to hatch. Thus the morphological effect of colchicine is apparently a non-specific one.

On the basis of external observations, the treated animals, in general, seem to fit into a graded series, the effect of the colchicine increasing with increasing concentrations. Colombo (1944) also noted this after colchicine treatment of the yolk plug stage of *Rana esculenta* and *Bufo vulgaris*, and Waterman (1940) noted it after colchicine treatment of fish embryos. It should be pointed out, however, that differential susceptibility exists among the series. This differential batch susceptibility is noted especially in the 0.1% concentration of group I in the present investigation. This is the concentration having the greatest number of series represented, and hence the greatest number of individuals. The series within this single concentration can be put into several different groups according to their degree of retardation. There is also individual susceptibilty within series. What seem to be apparent differences in susceptibility, however, may be due, in part, to the impossibility of selecting identical stages, as Dawson (1938) suggests.

The apparent difference in toxicity between the powder and the crystals may be due to either the form of the colchicine used or the age of the form used, the amorphous powder having been fresh and the crystals having been kept in the laboratory for at least two years. The latter cause is more probable since it is known that colchicine deteriorates with age—at least, if care is not taken to keep it in complete darkness (U. S. Pharmacopoeia, 1936).

It has been pointed out that the four-hour treatment was more effective in producing abnormalities than the one-hour treatment. Colombo (1947) also noted that the toxic effect of colchicine is proportional to the duration of its action when frog embryos are treated at the blastula stage or later, and Waterman (1940) has noted this effect on fish embryos.

Some of the externally visible abnormalties that have been described in the present paper also have been reported by Wolsky (see Colombo, 1944) and by Colombo (1944, 1947) after treatment of gastrulae and other stages with colchicine. These abnormalities include general retardation, microcephaly, acephaly, extrusion of yolk, and oedema (indicated by swelling of the ventral region). Macroscopic abnormalities described by Samartino and Rugh (1946) include meroblastic cleav-

age, exogastrulation, microcephaly, acephaly, stunting, distortion, wrinkling of body ectoderm, curvature of the body.

Many of the types of abnormalities produced in frog embryos by colchicine are similar to those produced by other environmental, and also by internal, factors. A number of these similarities has been noted earlier in this paper. The smooth-surfaced protrusions from the body wall is one type of abnormality described by Briggs (1941) following delayed fertilization. Oedema sometimes, at least, is associated with haploidy and osmotic imbalance (Rugh, 1941). The latter was noted by Piiper (1933) after treatment of frog embryos with cane sugar solutions, and by Baldwin (1919) and Rugh (1950) after x-irradiation of frog embryos. The "swayback" embryo and other types of abnormalities illustrated by Rugh (1941, abnormalities 80 and 84) as being found frequently in parthenogenetic or androgenetic tadpoles were noted after colchicine treatment in the present experiment.

A study of serial sections revealed that large irregular ventral spaces are often present in the yolk mass of those embryos succumbing to the toxic effects of colchicine. Probably these spaces are due to a progressive loss of yolk, as suggested by protruding yolk plugs and by exuding yolk in living embryos. A study of sections suggests that the infiltration of cells noted in the neurocoel and accessory cavities (table 3) represents the sloughed cells noted in the living material. These cells appear to have been caught within the neurocoel when the neural folds were closing to form the neural tube. Masses of cells in the neural canal have been noted after experimental procedures involving dinitrophenol (Waterman, 1939; Dawson, 1938) and after x-ray treatment (Solberg, 1938; Baldwin, 1919). In higher colchicine concentrations, the nerve cord is represented only by a mass of incompletely differentiated cells in which the neurocoel is absent. These conditions are similar to those found in embryos that have developed from over-ripe eggs (Witschi, 1930). In the same paper, Witschi described an excess of brown pigment granules in treated eggs (compare table 3 of present paper).

Table 3 indicates also that cells containing yolk were found in the notochord, giving the normally vacuolated notochord a more or less solid appearance in the higher concentrations. This also has been described by Colombo (1944), and a similar situation has been described in frog development after treatment with cane sugar solutions (Piiper, 1933). This latter paper also describes the gut as being partially blocked with yolk, as was noted in the present experiment, and by Hoadley (1938).

Failure of the heart to differentiate (see table 3) also has been noted by Solberg (1938) and by Baldwin (1919) after x-ray treatment; irregularities in heart form were mentioned by Waterman (1940) after colchicine treatment of fish embryos.

Histological results, in general, seem to indicate that organs are retarded progressively (or completely inhibited) as embryos are exposed to progressively higher colchicine concentrations, depending to a certain extent on their position along the embryonic axis. In the present investigation, the brain is the most markedly affected part of the central nervous system. The sense organs are inhibited progressively: the olfactory pit is absent in the least effective concentration used (table 3); the optic vesicles are inhibited in the next higher effective concentration; and the otic vesicles are inhibited markedly only in still greater concentrations. In the digestive system the pharynx is inhibited by concentrations in which the mid-gut is differentiated. Colombo (1944) notes that the differentiation of the pharynx and gills is related to the normality of the brain, their organization being poor if the brain is abnormal. This relationship is suggested in table 3, especially in the case of the gills. Solberg (1938), Colombo (1944), and Samartino and Rugh (1946) have pointed out that there is a fundamental difference between cells dividing without differentiation and those about to undergo differentiation. This is shown by their differential susceptibility to colchicine, to other chemical agents, and to x-ray treatment. Some of the effects of these external factors have been described in the present paper.

The exact mechanism by which colchine acts is unknown; as Colombo (1944) points out it does not produce a direct toxic effect but acts through individual cells. It is well known that colchicine acts on both plant and animal cells by inhibiting mitosis, which may result in polyploidy (Bergner, Avery, and Blakeslee, 1940; Allen and Creadick, 1937; *et al.*). Its action of preventing spindle formation by preventing the rise in viscosity which normally accompanies cell division has been demonstrated (Wilbur, 1940; Beams and Evans, 1940; *et al.*). The primary effect of colchicine, then, is on cellular action. Colombo (1947) points out that during cleavage and gastrulation colchicine acts primarily upon nuclei. At later stages he concludes that colchicine affects the utilization of yolk and interferes with water metabolism in the developing embryo. The former is suggested in the present investigation in which yolk granules have been seen dispersed throughout most of the organ primordia at the higher concentrations of colchicine. Also in support of this, Keppel and Dawson (1939) have described a progressive vacuolation in the uncleaved yolk mass of abnormal blastulae following treatment with colchicine. In some little understood way colchicine also seems to interfere with water metabolism not only for the single cell but for the embryo as a whole. Colombo (1944) points out that the pressure of imbibition produced in embryos treated with colchicine would impede gastrulation and by increasing the total water intake would produce oedema and enlargement of the pericardial cavity. Similar results have been obtained in the present investigation.

SUMMARY

The eggs of *Rana pipiens* were obtained by pituitary injections, separated into small groups, and a short time after fertilization incubated at 18°C. They were allowed to develop at this temperature until fixed, except for brief periods during which they were removed to room temperature for observations. Embryos were treated with colchicine when they were between Shumway stages 11 and 12 (late gastrula). They were fixed at Shumway stage 20 (hatching stage).

The results indicate that, within the limits of the experiments, colchicine activity is correlated with—

(1) temperature: at higher temperatures (22°-24°C in preliminary experiments) colchicine is more toxic than at lower temperatures (18°C);

(2) length of exposure: the longer the exposure to the drug, the greater the effect;

(3) concentration: the higher the concentration used, the greater the effect;

(4) either the form of colchicine used or the age of the drug used: either the amorphous powder is more toxic than are the crystals, or the length of time the crystals were kept before being used reduced their potency. The latter is more probable.

Mortality after colchicine treatment is correlated with concentration and length of exposure.

Some of the readily observable external effects of colchicine on frog embryos are general retardation and inhibition involving a disturbance of the cephalocaudad axis, microcephaly, oedema, persistent yolk plugs, and ectodermal enlargements.

Histological observations indicate that the degree of sensitivity of the organs studied is correlated with their degree of differentiation and to a certain extent with the position of the developing organs along the embryonic axis. There was some indication of polyploidy in that many of the nuclei appeared abnormally large.

It was noted that the abnormal effects produced by colchicine in *Rana pipiens* are similar to abnormal effects induced by other treatments, such as the action of dinitrophenol, cane sugar, x-ray, extremes of temperature, and delayed fertilization. It, therefore, cannot be said that any of the effects noted are specific for colchicine.

The exact mechanism by which colchicine acts is unknown. Its primary effect is a cellular one, for during cleavage and gastrulation it seems to act primarily on nuclei. At later stages colchicine disturbs the utilization of yolk and interferes with water metabolism in the developing embryo.

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NEW LAEVICEPHALUS FROM THE UNITED STATES

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In re-examination of members of *Laevicephalus* as limited by Oman in The Nearctic Leafhoppers, 1949, the following species were found to be new.

Parts figured inner ♂ gentalia, are not further described. All types are to be deposited in the Collection of The Ohio State University unless other deposition is mentioned.

I wish to thank E. S. Ross and H. B. Leech of the California Academy of Sciences for locating and lending a specimen of *L. pupa* (Van Duzee).

Laevicephalus inconditus n. sp.

Figures 16–18

Related to *L. minimus* (O. & B.) and *L tritus* Beamer, but with distinctly different aedeaga characters.

Yellowish green. Vertex with pair of oblique fuscous dashes just below apex. Elytra subhyaline, veins yellow, margined with fuscous toward apices. Face fuscous with 7 short pale arcs either side; sutures dark. Venter and dorsum dark medially to genital segments which are light.

Vertex in ♂ blunt and about equal in length to pronotum. Head wider than pronotum, side margins very short, rounded to straight hind margin. Elytra exceeding abdomen. Vertex in ♀ rectangular, longer than pronotum.

Genitalia. Value in ♂ longer than preceding segment, bluntly triangular, Plates wider than valve at base, two and one-half times as long as valve, broad, narrowing gradually to blunt appressed apices, uniseriate spine-like setae on margins. Elongate setose pygofer exceeds apices of plates by less than half their length. Last ventral segment in ♀ a little less than twice as long as preceding; posterior margin only slightly produced on median third, sides faintly sinuate, a short slit, about one-fourth length of segment at center, with a small rounded notch either side; central notched area and ovipositor except tip black or dark brown.

Length: ♂ 3 mm; ♀ 3.5 mm.

Close relationship to *minimus* and *tritus* is shown in genitalia of both sexes. The principal difference is in the form of aedeagus. In *minimus* both branches are long and separate from the shaft; in *tritus* they are mere stubs but retain the asymmetrical placement, and in *inconditus* the upper branch is united with the shaft but for its tip.

Collected in Texas by D. J. and J. N. Knull; ♂ holotype, allotype, 7 ♂ and 16 ♀ paratypes, Chisos Mts., July 17, 1946; 1 ♂ Culberson Co., Aug. 18, 1950; and Davis Mts., 1 ♂ June 8, 1939, 1 ♂ June 21, 1949, 5 ♂ and 4 ♀ July 2, 1940, 1 ♀ July 23, 1946, 3 ♀ paratypes and 10 specimens Sept. 20, 1938.

Laevicephalus curvus n. sp.

Figures 7–9

Resembling *L. tritus* Beamer and Tuthill, but smaller and with the aedeagus twisted.

Dark tan, eyes dark, face tan with pale arcs, apex of vertex with dark arc either side almost reaching black ocelli; dorsum but for tip, median part of abdomen, ovipositor and median part of segment above it black. Elytra semihyaline, veins yellow, bordered by dark smoky cell margins.

In ♂ length of head equal to that of pronotum and to width between eyes, bluntly rectangular. In ♀ head acutely angular, less than a right angle, one-fourth longer than pronotum and than width between eyes.

Genitalia. ♂, valve a small triangle, plates broad and long with sides almost straight narrowed to broad tips sharp on median side, exceeded by pygofer by half their length. Segment of ♀ as in *tritus*, half longer than preceding, sides rounded to median third which is produced,

with short slit at middle, and angular notch either side forming four teeth which are not as deep as in *tritus*.

Length. ♂ 2.5 mm; ♀ 3 mm.

Taken in Texas by D. J. & J. N. Knull: ♂ holotype, allotype and ♀ paratype, Gillespie Co., May 7, 1946; 2 ♂ Uvalde, June 26, 1949 and May 11, 1946; 1 ♂ Val Verde Co., May 24, 1948; 2 ♂ Benchley, April 30, 1941.

Laevicephalus longus n. sp.

Figures 1–3

Near *L. obvius* Knull, but longer. The largest species of the genus.

Greenish yellow, brighter yellow in ♂, front brown with pale arcs either side, vertex with curved brown arcs either side of apex fading toward ocelli, upper dark dash of face visible from above as straight mark between apex and dark ocellus; dorsum, median part of venter to last segment and ovipositor dark. Elytra semihyaline with yellow veins, apices faintly smoky.

In both sexes vertex bluntly rectangular, as long as pronotum at middle, elytra extending well beyond tip of abdomen.

♂ valve triangular, plates long, broad, narrowed very gradually to broad, blunt appressed tips, exceeded by spiny pygofer by half their length. ♀ segment produced on median third to rounded apex, no notch, sides sinuately excavated; produced part translucent so dark ovipositor shows through it.

Length; ♀ 5.5 mm; ♂ 4.2 mm.

♀ holotype, allotype and 4 ♀ paratypes, Huachuca Mts., Arizona, August 13, 1950; 4 ♀ paratypes, 1 ♀ and 1 ♂ specimen, August 19, 1950, D. J. & J. N. Knull.

Laevicephalus vannus n. sp.

Figures 4–6

Near *L. siclus* DeL. & Dav. but larger. There is variation within a species in Laevicephalus especially in the group in which the last ventral segment in the ♀ is produced convexly rounded, chiefly in constriction at sides and indentation or creasing at apex. Because of its larger size, although the segment is similar to that figured for *L. siclus*, and since no specimens have been seen which correspond exactly to *L. austrinus* DeL. & Dav., although it agrees fairly well with the rest of the description of that species, this is considered distinct.

Yellowish white with green tinge on pronotum and scutellum. Front with pale stripe down center, and alternating pale and brown arcs. Curved fuscous arcs extend from below apex of vertex to ocelli. In ♂ a pair of dashes either side just above base midway between median line and eyes. Dorsum dark, venter dark in middle of segments, darker in ♂. Ovipositor a little darker than pygofer, especially on sides. Elytra semihyaline with yellow veins. In ♂ head as long as pronotum and as long as width between eyes; in ♀ longer than pronotum; apex of head bluntly rectangular.

♂ valve small, triangular with apex a small rounded lobe. Plates as broad at base as last ventral segment, wider than valve, extending beyond tip of valve twice its length, narrowing gradually to blunt tips separated slightly at apex, exceeded by spiny pygofer less than half their length. ♀ last ventral segment as long at sides as preceding segment, then excavated and median third roundly produced with faint indentation at apex. Top of this and preceding segment appear creased at middle.

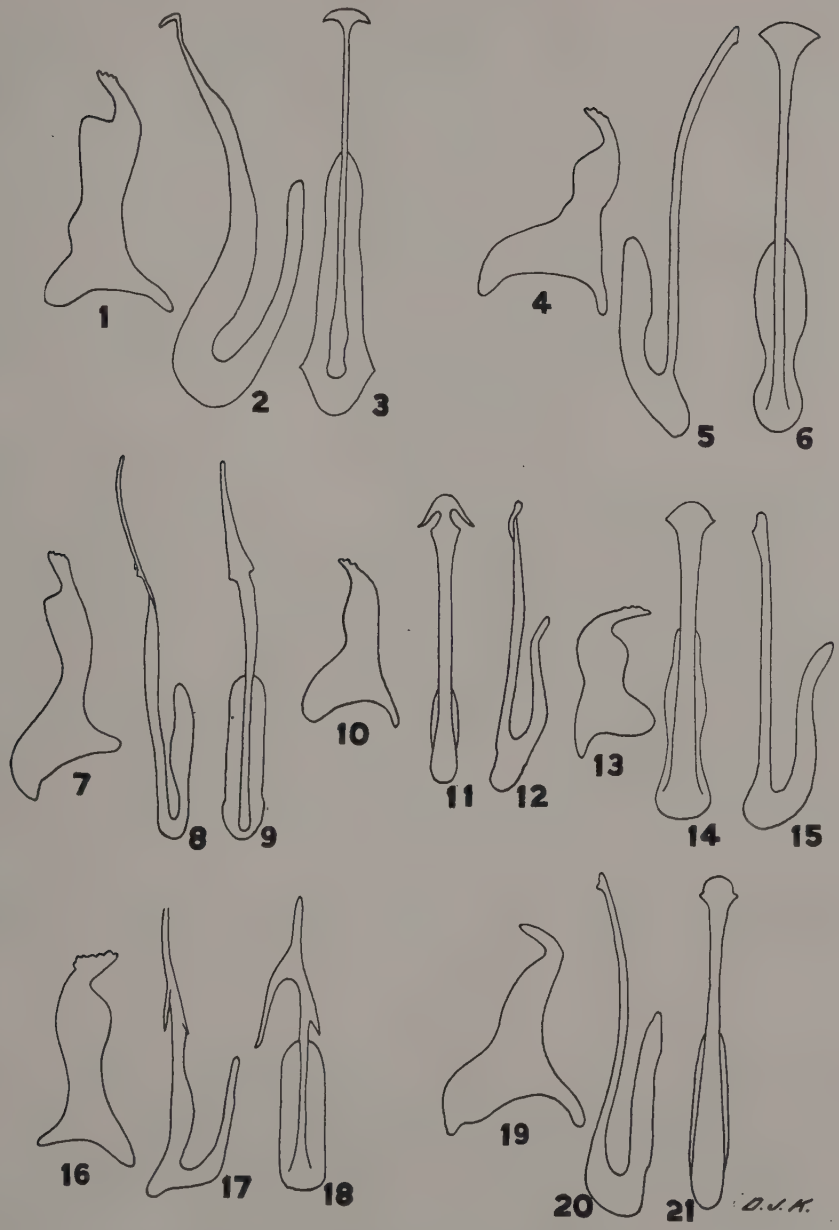
Length: ♂ 3.8 mm; ♀ 4 mm.

♂ holotype, Davis Mts., Tex., May 9, 1941; allotype and 2 ♀ paratypes, Davis Mts., Tex., June 2, 1951; 1 ♂ Val Verde Col., Tex., June 14, 1949; collected by D. J. & J. N. Knull. 6 ♂, 7 ♀ paratypes, Buffalo, S.D., June 28, 1947; 1 ♂ Martin, S.D., Sept. 11, 1948; 2 ♀ and 2 ♀ Logan, N.M., Aug. 28, 1950, collected by H. C. Severin. Paratypes in Dr. Severin's collection.

Laevicephalus exiguus n. sp.

Figures 19–21

Greenish yellow, darker on disc of pronotum; brown arcs from either side of apex of vertex not reaching black ocelli, double dash dark brown near base either side midway between black median line and gray eyes, area between arcs and dashes brown tinged; two narrow brown lines



(Inner ♂ genitalia all drawn to the same scale)

1– 3. Style, lateral and ventral view of aedeagus, *L. longus*.
4– 6. Style, lateral and ventral view of aedeagus, *L. vannus*.
7– 9. Style, lateral and ventral view of aedeagus, *L. curvus*.
10–12. Style, ventral and lateral view of aedeagus, *L. salarius*.
13–15. Style, ventral and lateral view of aedeagus, *L. tantalus*.
16–18. Style, lateral and ventral view of aedeagus, *L. inconditus*.
19–21. Style, lateral and ventral view of aedeagus, *L. exiguus*.

below double dashes on anterior half of pronotum faintly indicated. Face yellow with pale median strip, either side of which are alternating pale and dark arcs, sutures and dot on outer margin of lora dark. Body dark except genital segments and lateral margins; elytra semihyaline with pale veins.

♂ head shorter than width between eyes and a little shorter than pronotum, broadly rounded, more than a right angle. ♀ head a little longer than pronotum, but shorter than width between eyes.

♂ valve small, triangular, blunt tip; plates narrowed on outer half, inner margin straight, sharp-tipped, exceeded by spiny pygofer by about one-third their length. ♀ last ventral segment as long as preceding, at sides, produced a little on middle third, entire.

Length: ♂ 2.8 mm.; ♀ 3.2 mm.

Collected in South Dakota by Dr. H. C. Severin: ♂ holotype, allotype, 1 ♂ and 2 ♀ paratypes, Badlands, Interior, June 15, 1948; 1 ♂, 1 ♀ Fox Ridge, June 28, 1947; 1 ♀ Phillip, Aug. 7, 1948; 4 ♀, Buffalo, 1 June 27, 1950, 3 Sept. 10, 1948; 4 ♀, Highmore, 1 June 14, 1948, 2 June 23, 1950, 1 Aug. 14, 1949. Paratypes in Dr. Severin's collection.

Laevicephalus tantalus n. sp.

Figures 13–15

Near *L. exiguus* n. sp. in character of inner ♂ genitalis but smaller, with very blunt head in ♂ and produced head in ♀.

Greenish yellow with two large patches either side of apex, not touching ocelli, a streak against each eye, and an elongate oval enclosing pale spot, midway between eyes and central dark line from base half way to apex, brown. Faint indication on middle of pronotum of two narrow longitudinal stripes. Face bright yellow, especially toward top, center pale, with brown and light arcs irregular. Elytra almost clear, veins yellow. Dorsum black but for edges and pygofer, venter dark medially to last two abdominal segments.

♂ head broadly rounded, only silghtly longer at middle than against eye, three-fifths as long as pronotum and shorter than width between eyes. Valve broad, short and rounded, plates wider than valve, narrowed gently on outer half then produced to blunt, appressed tips, exceeded by pygofer by almost one-third their length. ♀ head produced, rectangular, longer than pronotum and longer than width between eyes. Marking as in ♂ but elongate. Last ventral segment longer than preceding, produced slightly, with margin faintly sinuate, and with no median notch or indentation.

Length: ♂ 2.5 mm; ♀ 2.7 mm.

Collected in South Dakota by Dr. H. C. Severin, types include: ♂ holotype and 2 ♂ paratypes, Phillip, Aug. 7, 1948; 1 ♂ Kimball, June 17, 1947; 1 ♀ allotype, Badlands, Interior, June 15, 1948. Paratypes in Dr. Severin's collection.

Laevicephalus salarius n. sp.

Figures 10–12

A pale species near *L. obvius* Knull but smaller.

Yellow, darker on disc of pronotum, usual brown arcs reduced to a spot either side of apex; face yellow with arcs very faint, sutures black. Dorsum black, venter and lateral margins as well as dorsum of pygofer light, sides only of ovipositor darkened.

♂ head blunt, considerably shorter than pronotum and then width between eyes; ♂ head a trifle longer at middle than pronotum, and than width between eyes, bluntly produced, more than a right angle.

♂ valve small, irregularly triangular, plates long, straight-sided, narrowing only slightly outside to blunt tips, exceeded by pygofer by less than half their length. ♀ segment twice as long at middle as preceding, produced from middle in a short rounded lobe slightly sinuate over ovipositor.

Length: ♂ 2.6 mm; ♀ 3.3 mm.

Taken in Utah in 1950 by Dr. H. C. Severin as follows: ♂ holotype, allotype, 4 ♂ and 4 ♀ paratypes, Great Salt Lake, Aug. 26; 1 ♂ Bryce Nat. Park, Aug. 30; 2 ♂ Marysville, Aug. 26; and 1 ♀ Manti, Aug. 25. Paratypes in Dr. Severin's collection.

DISCUSSION

In studying this genus it was found that the following species are readily recognized from external characters in both sexes: *acus* (S. & Del.), *longus* Knull (on basis of size), *melsheimerii* (Fitch), *monticolus* (G. & B.) *poudris* Tuthill, *sylvestris* (O. & B.) and *unicoloratus* (G. & B.)

Three groups of species show close interspecific relationship. One, the Sylvestris Group, includes *acus* (S. & Del.), *pravus* Delong, and *sylvestris* (O. & B.), all with the aedeagus S-curved in lateral view. ♀s of *sylvestris* and *pravus* cannot always be separated. Only a few specimens in large series of *pravus* examined corresponded in character of last ventral segment to DeLong's (1948) illustration for it.

In the Minimus Group are: *curvus* Knull, *minimus* (O. & B.), *inconditus* Knull, and *tritus* B. & T., all with aedeagus asymetrically branched and last ventral segment of ♀ incised at middle, and indented either side of incision, forming four teeth. Depth and form of these incisions is variable within a species and it is not always possible to separate them from ♀s alone.

The chief difficulty arises in the Parvulus Group in which it is necessary to examine internal ♂ genitalia for specific determination, and unassociated ♀s cannot be reliably placed on morphological characters, at least for this author. To this Group in which there is usually a pronounced difference between shape of heads of ♂s and ♀s, the ♀ heads being more bluntly rounded, belong the following in which ♂s are known: *aridus* Oman, *canyonensis* Knull, *exiguus* Knull *hamatus* Beamer, *longus* Knull, *obvius* Knull, *parvulus* (Gillette), *paulus* Knull, *salarius* Knull, *tantalus* Knull and *vannus* Knull. The following which were described from ♀s and in which ♂s are not known probably may be referred to this Group: *austrinus* DeL. & Dav., *bocanus* Oman, *convergens* (DeL.), *pupa* (Van Duzee and *siclus* Del. & Dav.

Vafer Knull is near the Parvulus Group, but lacks the terminal broadening of the aedeagus which is characteristic of it.

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Fatigue of Metals. *R. Cazaud.* Translated by *A. J. Fenner.* Philosophical Library, Inc., New York. 1953. xiv+334 pp. $12.50.

This book by Dr. Cazaud is about the failure of metals under oft repeated stresses. It deals with many phases of fatigue testing including testing machines and the effects of physical variables on the results. The opening chapter is a brief history of the subject and there follows an excellent description of the physical and visual characteristics of fatigue failures with some very fine photographs. The book is a good resumé of factual knowledge and is heavily documented with references, curves, tables and the results of experiments, many of them the author's own work.

The editing and presentation are somewhat ragged. Typographical errors are noted and chapter headings do not always define clearly the content of the chapter. Theories of the mechanism of fatigue are recounted in too hasty a manner and the author seems timid about using the mathematical and thermodynamic approaches with which current literature is crowded.

However, being non-mathematical, the book can be read by mill foremen, shop operators, and draughtsmen and yet it is not without value to the more advanced student of physical testing.

The original book, published in 1948, is ably translated by Mr. Fenner. It reads easily and interestingly and except for the use of metric values one would not suspect its foreign origin.

J. O. Lord.

AN ADDITIONAL RECORD OF THE LONE-STAR TICK, *AMBLYOMMA AMERICANUM* (LINN.) IN OHIO

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Amblyomma americanum (Linn.), the lone-star tick, is easily identified by the solitary white spot on the posterior margin of the scutum of the female. Possessing long, piercing mouth parts, *A. americanum* can inflict painful injury, and in addition is a vector of Rocky Mountain spotted fever and a suspected vector of American Q fever, tularemia, and Bullis fever.

Cooley and Kohls (1944) in their monograph on the genus *Amblyomma* in the United States report that *A. americanum* is most abundant in Texas, Louisiana, Oklahoma, Arkansas, and Missouri, but state that there is little published information concerning its distribution. In Ohio this tick has been reported previously only twice, these records together with an additional recent collection record being summarized as follows:

The first record of this tick occurring in Ohio was reported by Miller (1925) who gave no collection data other than to state that his specimens came from Bellefontaine. Riley (1944) reported the collection of a female *A. americanum* from a human at Jackson, Jackson County, Ohio, April, 1939.

An additional record of *A. americanum* was obtained when a tick collected by Don Tennant from a baby girl on May 11, 1952, at Mt. Gilead, Morrow County, Ohio, was brought to the Department of Zoology and Entomology, The Ohio State University. This tick was identified by the writer as a female *A. americanum*, and will be placed in the insect collection of the Department of Zoology and Entomology, The Ohio State University.

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The Science of Biology. *William C. Beaver.* The C. V. Mosby Co., St. Louis. Fourth edition, 1952. 895 pp. $5.85.

Biologic Science in Laboratory and Field. *William C. Beaver.* The C. V. Mosby Co., St. Louis. Fourth edition, 1952. 253 pp., paper bound. $3.50.

These volumes are designed as a text book and laboratory-field manual, respectively, for a year's course in biology. The use of the scientific method as a means of observing and recording facts, formulating conclusions, and deriving principles from them is stressed. The author feels that illustrations need not be included for the entertainment of the student, but rather to assist him in gathering and evaluating facts scientifically. Nevertheless there are 375 illustrations in the text and several in the manual, including some outline drawings to be completed by the student.

A BRIEF EVALUATION AND DISCUSSION OF AN IMPORTANT DEVICE IN HIGH SCHOOL SCIENCE TEACHING[1]

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"The project method of teaching" is a dilapidated term. By whatever name, though, a rose is still a rose. And enticing high school students to make models, equipment, displays, or to do a bit of research that will interpret or illustrate or demonstrate the topic of discussion, is considered by authorities today the most successful way of teaching high school science. The method not only makes science more interesting and more easily understood; it lets the teacher reach the exceptional students at both scholastic ends of the class as well as the majority in the middle; it is a very effective way to detect scientifically talented youth.

In order to encourage this method and to seek the talented, "science days" or "science fairs" have sprung up throughout the United States.

Section J of the Ohio Academy of Science sponsors the Ohio Junior Academy of Science and stages annual district science days at six universities as well as an annual state science day which is held at the annual meeting of the Ohio Academy of Science.

These science days are unique in that while the final state science day features the best projects from the six districts and the most talented students are the participants, many *district* science-day projects are exhibited by the less endowed students, occasionally even a retarded student. He does not receive a superior rating, but he gets the recognition and warm satisfaction characterizing the science day atmosphere.

Everyone receives a rating indicated by a certificate. Teachers find that slow students are as proud of their "good" ratings as the best students are of their "superior" ratings. One retarded student told his teacher that he had shown his certificate to everyone on his street and that his mother had telephoned several relatives about it!

There are no large awards to be won in these fairs. Tuition scholarships to certain universities of Ohio are given to about twenty seniors. A fifty-dollar prize is offered the school doing the best work. The most important award is that obtained by every participant—the joy of associating and conversing with others interested in one's own creative work and the self-confidence and self-respect gained by the experience.

Highly favorable and enthusiastic remarks come from the students. They enjoy seeing what other students have done. They get new ideas. They learn science. They become acquainted with the many ramifications of science through one another. They like the college atmosphere where the fairs are held.

It is quite surprising sometimes to see what these youngsters will dream up. It is startling to see how and to what extent an idea is developed by a ninth grader. It is interesting to find out what started a project idea.

A member of the National Honor Society, David Dowds of Urbana, received a superior rating for the photomicrographic work shown in figure 1. About two years ago a series of photomicrographs in a magazine caught David's interest, photography being his hobby. Shown here is a number of tissue micrographs he made with his camera and microscope. He is anxious to do a series of molds and a series of crystals next. Dave plans to major in biochemistry at Ohio State

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University next year. He says that Science Day definitely inspires interest in science in youth.

With the help of an old carpet sweeper, slightly dissected, Edward White, ninth grader at Jackson High School, melts iron in a cupola which he claims is one-half inch smaller than "the world's smallest cupola" (fig. 2). It took only three months

TABLE 1

Numbers of participants in State Science Day, April, 1953, by districts

District	Number of Students	Number of Towns	Number of Schools	Number of Projects	Percent of Total Projects
Northwest	82	20	25	66	31
Northeast	47	17	23	42	19
East	11	5	6	10	5
Central	51	8	16	51	24
Southwest	33	9	14	25	11
Southeast	22	7	9	21	10
Totals	246	66	93	215	100

TABLE 2

Percent of kinds of ratings achieved by each district

District	Percent of Superior Ratings	Percent of Excellent Ratings	Percent of Good Ratings
Northwest	44	38	18
Northeast	31	48	21
East	30	40	30
Central	37	43	20
Southwest	64	20	16
Southeast	38	57	5
Total	41	41	18

TABLE 3

Projects and ratings by subjects

Subject	Number of Projects	Percent of Total	Percent Superior	Percent Excellent	Percent Good
Botany	25	12	48	36	16
Chemistry	57	26	37	51	12
Geology	10	5	20	30	50
Math	3	1	33	67	
Physics	78	36	44	37	19
Zoology	38	18	45	37	18
Home Ec.	2	1	50	50	50
Conservation	2	1		50	

after his dad suggested the idea to get things smelting. He hopes to make an iron casting of the Boy Scout emblem this summer with his "superior" project. A profession in foundry work beckons Ed who relaxes with music—singing and band work. He anticipates going to college at Antioch. He is quite impressed with the amount of science he learned at Science Day.

FIGURE 1

FIGURE 2

Although Richard Pavely is only a ninth grade general science student, he is already well into the science of chemistry as shown by his "superior" rated project, fig. 3. He has rigged up a neat, compact display of his knowledge of electrochemistry. His heart is set on some sort of engineering profession and he is grateful to Science Day for enabling him to get a bird's-eye view of projects in many different scientific fields. Dick, who sticks to the honor roll, is a student at Purcell High School in Cincinnati.

The labs in the Botany and Zoology building at Ohio State University were cramped quarters on Science Day, but that didn't prevent Jo Ann Brodbeck from scanning the skies with the "superior" reflecting telescope she had spent 100 hours making. It is shown in fig. 4. This Archbold High sophomore has really fallen for astronomy in a big way. Last year she made an umbrella planetarium,

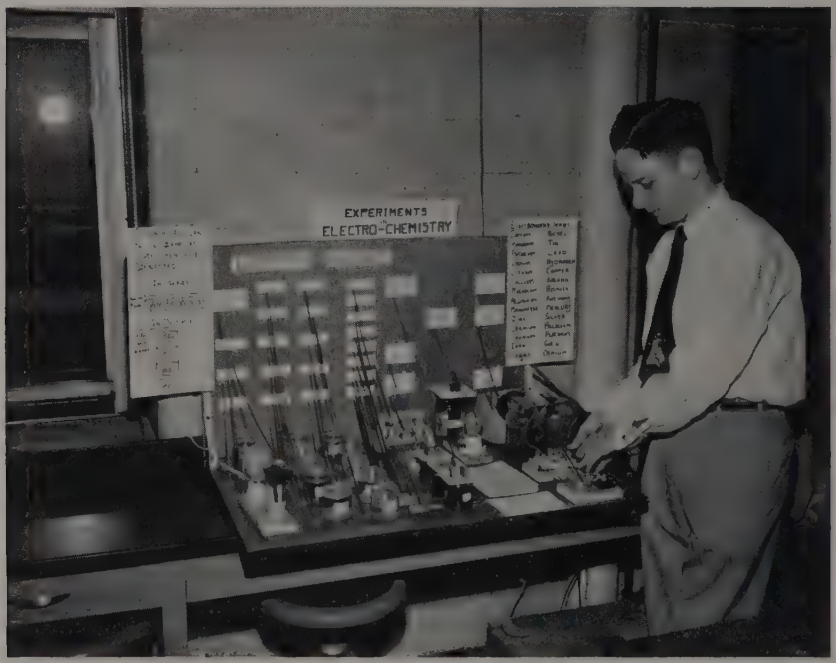

FIGURE 3

this year the telescope, and next year—well, wait and see. She's quite versatile too. She has taken state honors with her flute, took the state scholarship test in English, is pledged to the National Honor Society, and hopes to become a school teacher! Either Capital University or Wittenberg will be the lucky winner in '55.

This is merely a sampling of the splendid work displayed at the Academy Science Day this past year. Tables 1, 2, and 3 give a summary of this event. The 246 participating students represent only a part of those who exhibited projects in the district science days; and participants in the district science days represent in most schools only a small part of those who actually made projects.

Project making, I think, is the most important tool a science teacher has. A class period spent in discussing a student's project recently made is more interesting and instructive than anything I know. Projects may be assigned; they may be suggested; they may be the student's own idea. Projects can be individual

things for the introvert; they can be group undertakings for the social-minded. Projects can be simple or complex. The retarded child will enjoy making several simple projects. The superior student will think up something complex and will make it more complex before he stops, if he ever does stop. Some get their dads and even their mothers to help them. Students give each other ideas. Projects bring out preseverance in students. They are enjoyed equally by girls and boys.

FIGURE 4

Many high school teachers feel that our science days present an essential and unique opportunity in creating interest. It is not a contest; it is a very satisfying experience for all children, not just the best. "The project method" as a term is a cliché; as overt reality, it is unrivaled.

SPECIATION WITHOUT ISOLATION

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Often we find in older discussions of evolutionary processes, statements possibly correct according to then existing information, but which subsequent accumulation of knowledge has rendered doubtful. In scarcely any instance has there been more frequent and rapid shifting of ground than in treatment of the general proposition of isolation.

Darwin recognized the importance of separation, both in artificial and natural selection, but he did not especially emphasize it. Moreover, he did not agree with Moritz Wagner "that migration and isolation are necessary elements for the formation of new species" (?1912, p. 90). In fact he could not well do so without depreciating just so much the force of the arguments for natural selection. The latter is in fact only a form of isolation (by allegedly restricting breeding to similar favorable variants) as recognized by Romanes, and every admission as to the importance in evolution of physical and other forms of isolation restricts the field of natural selection. It is not my intention to review the literature of isolation as a factor in evolution but rather to comment briefly on various phases in which the idea has been presented. Geographical isolation as a necessary factor to the formation of varieties and species of organisms was an early postulate. This crude generalization could not be approved for exceptions to it were part of the knowledge of every naturalist. It is well known both that isolation is not necessary to coexistence of related species, and that isolation does not necessarily result in differentiation. Then in turn ecological or seasonal separation were appealed to, and these not covering every case, finally physiological and morphological isolation were assumed as necessary to permit the development of specific differences among forms obviously not separated in any spatial sense.

It is unnecessary to cite cases typical of those that caused these changes of argument on the part of adherents to the hypothesis that some form of isolation is a necessity for speciation. It will serve to consider a few instances in which all of the suggested forms of isolation except the morphological or physiological are excluded. I may allude to the 8 species, more or less, of *Antennaria* that inhabit dry open places in the Northeastern States, to the same number of species of *Bidens*, all marsh plants of the same region. In Florida swamps there are 8 species of *Tillandsia* epiphytic on trees and 10 of *Utricularia* living in ponds and ditches. For a western example, there are 13 species, more or less, of *Eriogonum* in the Mojave Desert. Hodges (1936) has reported the identification of 32 species (besides several varieties) of *Fusarium* growing on sugar beets in storage at Syracuse, N. Y.

Woltereck (1933, p. 515) referred to "the fifty, about, endemic *Haplochromis* fishes living pell-mell in Lake Victoria, which, with one exception, are all unique, or the three hundred gammarids in the bottom mud of Lake Baikal, or the twelve to twenty species of cyprinid fishes in Lake Lanao; we cannot deny some internal causes of such differentiations or explosions, because they are not fifty or three hundred or twenty different localities (environments) within these lakes. And if we consider, for instance, the hundreds of very different Radiolaria or Tintinnidae or Coccolithophorides living pelagically under identical conditions in the same region and the depths of the Pacific Ocean, we face again the same necessity of admitting immanent capacity and trend to change."

Kofoid (1907, p. 503) stated that 50 species of *Coscinodiscus* occurred in Ant-

arctic plankton taken on one expedition between depths of 60 and 40 meters, and further, that in his own studies of the genus *Ceratium* of the dinoflagellates "in the plankton of the Pacific immediately at the surface, that is in the upper 2–3 meters, it is no unusual thing to take 25–30 species in a single 14-inch net."

It can hardly be argued, with reference to these cases, that there are niches in the necessarily very uniform environments named sufficient to accomodate such a multiplicity of species, but it can be postulated, although unreasonably so, that these species originated in spatially separated environments and congregated later. As a crucial case in which even such a far-fetched doubt cannot intrude, I would cite the tiny leafhoppers of the genus *Erythroneura* of which there are already known from the eastern United States about 50 species that live upon the leaves of grape. Several species so distinct that immature as well as mature examples can be recognized at a glance, as well as others more difficult of separation, can be simultaneously collected from a single plant, and individuals swarm.

Now in a case of this sort where isolation, if it exists at all, must be off a physiological (intersterility) or morphyological type (differentiation of the genitalia), it would seem that it is not isolation that permits the species to develop but rather that development of physiological or morphological differences have brought about whatever kind of degree of isolation that does exist. In other words, crowding in a uniform environment would provide such a free opportunity for interbreeding that speciation would be prevented except for intervention of some internally developed mechanism for preventing free crossing.

In such cases as that of the grape leafhoppers, and the example is merely extreme, not unique, it is clearly out of the question that specific characters could be developed by a process of selection of beneficial variations, for the organisms continuing to live together under identical conditions, there could be no advantage in merely being distinct. So far as advantage is concerned, such groups of species could just as well be one. If survival of the fittest ruled, there would be only one kind of grape leafhopper on the leaf at the same season, not several. Certainly in a stable environment an organism can be fittest only by being superlatively itself, not by diverging. Such examples are opposed to Darwin's postulate of divergence being promoted by natural selection as an advantage enabling occupation of new range, for in these instances divergence has clearly occupied without spread to new environment. The existing diverse species have manifestly developed together in identical environment. The picture presented is of organisms in the grip of an evolving power that brings about speciation without regard to environment.

The phenomenon probably may be taken as illustrative of what we shall find in many groups when their specific differentiation is worked out according to the most discriminative methods. Previously recognized specific groups will prove composite and we shall accordingly find numbers of species in environmental niches so uniform as to rule out the influence of external factors upon speciation.

Close analysis of groups has characterized modern work especially in insect taxonomy and the use of genitalic structures for separation of species has become increasingly prevalent. Peterson (1905, p. 224) has concluded from his work in this direction (upon butterflies) that "specific divergences have their origin in morphological differentiations in the sexual apparatus, which led to physiological isolation of groups of individuals and further that in the course of their isolation, indifferent and superficial characters by restricted breeding, become specific."

The tenor of the argument is that in cases really testing the validity of the isolation hypothesis, the origin and maintenance of characters preventing intercrossing has provided the degree of isolation necessary to the existence of closely related species in identical environment. Hence, speciation brought on isolation, not the reverse. The process is orthogenetic evolution and it undoubtedly prevails even in cases where isolation is more tangible, but has been neglected as an

explanation while other theories were having their day. Although endless discussion of external factors and their influence has served greatly to delay recognition of the fact, organic evolution clearly is in the main an innate process.

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About two-thirds of this excellent book is devoted to igneous petrology. Much of this material deals with the associations, petrological relations, and origins of various rock groups. It treats not only the concepts of magmatic differentiations but also the relations that may exist among environment, sedimentation, orogeny, and the origin of igneous rocks.

The part devoted to metamorphic petrology treats chiefly (1) the principles governing metamorphism and the formation of metamorphic mineral assemblages; (2) the characteristics of metamorphic facies and, in less detail, the role of metasomation and metamorphic differentiation; (3) the principles governing chemical adjustment of solid rocks to metamorphic conditions; and (4) closes with a discussion of metamorphism in relation to magma and to orogeny.

About one-fifth of the book is devoted to a consideration of the principles of physical chemistry and thermodynamics, about equal space being given to (1) principles of chemical equilibrium applied to rocks, (2) crystallization of igneous minerals, and (3) the principles governing chemical adjustment of solid rocks to metamorphic conditions.

References are given only in footnotes and are selected chiefly from modern material easily accessible. Extensive references to older literature are available in the papers cited.

The entire treatment is modern, and brings together important concepts regarding the origin and evolution of igneous and metamorphic rocks, and their possible relation to molten material and to transformations in the solid state. This book is one of the most important and useful reference books available, but for most profitable use the reader needs considerable background in chemistry, mathematics, and petrography. Also, it is an excellent but rather difficult text for graduate students. Thus it fulfills well the purpose for which it was written, "the use of advanced students, research workers, and teachers in the field of petrology."

CARL A. LAMEY

COMMUNAL INTERESTS CRUCIAL TO MARITAL ADJUSTMENT

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About fifteen years ago, Kirkpatrick (1937) stated the following hypothesis: "Community of interests between married persons is an important factor in marital adjustment, and a measure of a community of interests may be a relatively innocuous index for measuring adjustment and for predicting the success of a relationship between two persons of the opposite sex" (p. 134). Other researchers (Taves, 1948; Frumkin, 1952) have substantiated the validity of this hypothesis, i.e., they have proven that a community of interests can be and is an index of marital adjustment.

To measure the community of interests in marriage adjustment Kirkpatrick constructed what today is called *The Kirkpatrick Scale of Family Interests*. Simply stated, this scale consists of 60 interest items duplicated on two single sheets of paper. On the first sheet the respondent is requested to check those interests which he enjoys engaging in as personal interests. On the second sheet he is requested to check all those interests which he enjoys engaging in with his spouse. The adjustment score is readily obtained by dividing the number of personal interests by the number of personal interests which are also mutual interests, i.e., the double-check items.

A question is raised concerning the interest items selected by each spouse as communal interests. Which are the most important communal interests in the light of social change? More specifically, how do social scientists, teachers, students, and other educated persons feel about the significance of these interests to marital adjustment?

METHODOLOGY

In order to get a rating of the significance of the interest items for marital adjustment, i.e., significant in the way an interest may differentiate the well-adjusted from the unadjusted spouse, in the Kirkpatrick scale, 52 judges representing the sociology and psychology departments of the Ohio State University were asked to rate each interest item on a three point scale weighted as follows: S, significant, (equal to 3); M, moderately significant, (equal to 2); and I, insignificant, (equal to 1). By having the letters S—M—I— next to the numbered interest items, the judges had only to circle the letter which represented their choice. The directions were as follows: "In relation to marital and family adjustment certain activities participated in by *husband and wife together* seem to be more significant and crucial to *success* or *failure* than other activities. Please circle at the left of each activity the rating which, in your opinion, represents the significance of that activity in marital and family adjustment. S—significant, M—moderately significant, I—insignificant."

After all 52 judges had rated each interest item in the scale the mean score for each interest item was recorded and each interest item arranged according to this mean score in descending order. The top ten and the bottom ten were considered as those interests most *significant* and *insignificant* to marital adjustment.

FINDINGS

It is surmised that the judges are persons of fairly good background, intelligence, and understanding, and that they are fairly well qualified to evaluate the sig-

nificance of these interests to marital adjustment. In table 1, we can examine the results of their judging of the Kirkpatrick interest items.

By using the first and last fifteen interest items of the Kirkpatrick scale and randomly dividing the judges into two equal groups it was possible to compute a Spearman rank-difference correlation to determine the reliability of the judges' ratings. The correlation between mean significance ratings of the two random groups of judges was found to be 0.93. More than just a consistency between judges, this points to a consistency in which is agreed to be the most important, most significant mutual interests affecting marital adjustment. In other words there seems to be a consensus of "experts" as to what factors are associated with a successful or unsuccessful adjustment in marriage.

More than just a selection of factors associated with a successful or unsuccessful marriage, these selections represent and reflect the whole process of social change relevant to all spheres of life. These selections of *significant* and *insignificant* interests represent a new conception of man and a new conception of marriage and the family. Bernard (1933) has said that one of the social functions of marriage in our culture is the "serving of the primary group needs of the personality" (p. 95). She also added that "a marriage which builds and stabilizes

TABLE 1

The most significant and most insignificant interests of the Kirkpatrick scale according to the 52 judges

Significant Interests	Mean	Insignificant Interests	Mean
Demonstrating affection	2.88	Reading essays aloud	1.19
Planning for the future	2.86	Smoking	1.21
Planning saving or investment	2.84	Having picture taken	1.28
Training children	2.78	Reading poetry	1.34
Planning family budgets	2.75	Listening to the radio	1.40
Making plans for children	2.71	Gardening	1.42
Discussing intimate personal experiences	2.57	Explaining moods	1.51
		Buying books and magazines	1.53
Confiding worries	2.57	Discussing scientific topics	1.53
Playing with children	2.55	House cleaning	1.55
Discussing work done outside the home	2.48		

and integrates the personalities of its members is successful; one that disintegrates or represses their personalities or causes conflicts in them is unsuccessful" (p. 95).

Burgess and Locke (1945) point out: "In adjustment, as compared with other methods of judging marital success, the emphasis is upon consensus, upon the mutuality of interests, and upon the joint participation in activities" (p. 444).

Harper (1949), Nimkoff (1947), Duvall and Hill (1945), Winch (1952), and others agree with Bernard, and Burgess and Locke that "the modern family has only a few major functions not adequately provided by other institutions: namely, (1) love and companionship between husband and wife; (2) reproduction; and (3) protective and affectional services for pre-school children" (Harper, 1949, p. 68).

Winch (1952, p. 447) considers the following functions as most significant to the modern family: "economic, status-conferring, reproduction, security-providing, and even, to some degree, socializing."

Thus it can be seen that the 52 judges in our study and the family sociologists previously mentioned are in close agreement concerning the communal interests most crucial to marital adjustment. An examination of table 1 confirms this statement. Among the ten most significant interests chosen by the judges were

those of "demonstrating affection," "planning for the future," "planning for saving or investment," and "training children." It should be noted that the judges also agree that some of the traditional functions of the family are now less important to a successful marital adjustment than are the new functions of the modern family. Thus the judges mentioned among the least significant interests those of "house-cleaning," "gardening," and "reading essays aloud."

SUMMARY AND CONCLUSIONS

In this study we have tried to find out how a group of competent and educated persons would judge certain communal interests as related to success or failure in marriage. We found that these judges rated these interests in a way which was consistent with social change as it affects our conception of man, and our conception of marriage and the family. We found also, that leading family sociologists were in close agreement with our findings, i.e., our judges' ratings of communal interests crucial to marital adjustment. Thus we may conclude that there is not only a consensus of experts (of family sociologists) concerning what communal interests are crucial to marital adjustment but there is also a consensus among learned persons (not experts) concerning these interests.

What are the implications of our findings for persons who are now concerned with applying knowledge that is consistent with social change? It means clearly that professional persons working with the family must view these new functions of the family in relation to traditional functions. They must be cognizant of the inevitable conflicts which result from contradictory modern and traditional attitudes concerning the most important functions of marriage. Divorce courts will testify to the fact that the judges' selection of *the most crucial communal interest*, i.e., that of "demonstrating affection," is an interest which is crucial in modern marriage. They may also assert along with the 52 judges that the affectional function of modern marriage supersedes in importance the communal interests related to maturity. Yet we know that maturity is an extremely important factor in successful marital adjustment.

SUGGESTIONS FOR FURTHER RESEARCH

On the basis of the findings in this study the writer makes the following sug gestions for further research:

1. The significance of any particular communal interest must be assessed in relation to the social interaction taking place in a particular marriage, and in relation to the social matrix in which this marriage occurs. Thus, research that seeks to correlate actual marital adjustment in relation to particular communal interests is needed before we can say that any one communal interest is more crucial to success or failure in marriage than any other particular communal interest.

2. Perhaps we take too much for granted concerning the consensus of our 52 judges and family sociologists as to the importance of communal interests to marital adjustment. Thus, the writer suggests that further studies be done in order to determine scientifically what the actual relationship of communal interests is to other factors associated with marital adjustment.

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REDUCTION OF THE CLAVICLES IN THE MESOENATIDAE, WITH SOME REMARKS CONCERNING THE RELATIONSHIP OF THE CLAVICLE TO FLIGHT-FUNCTION IN BIRDS

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During recent studies on the arteries in the neck and thorax of the Mesoenatidae, it was observed that the clavicles are reduced to a pair of extremely small bones which articulate with the clavicular face of the head of the coracoids.

In discussing the taxonomic position and some features of the anatomy of *Mesites*, Beddard (1898) stated, ". . . The furcula is quite degenerate." Stresemann (1927–1934), apparently following Lowe (1928) stated, "Keine Spur einer Furcula." Both statements are vague and open to interpretation. According to Lowe (1924, p. 1148), "There is not the faintest indication of a clavicle in *Mesites*." This is not the true condition, however, and it is well to clarify this matter, and to give a brief account of the actual condition as found in single specimens of each of the two species of *Mesoenas* (*variegata* and *unicolor*) and in two examples of *Monias benschi*. A single alcohol-preserved specimen of each, and, in addition, a complete skeleton of *Monias*, were studied from the anatomical collections of the American Museum of Natural History and the U. S. National Museum. In each case the clavicles were carefully measured.

The clavicles, in each of the three species, are reduced to small rudimentary or vestigeal bones which represent only the head of the clavicle or epicleidium.

TABLE 1

Measurements of the os claviculum *in three species of Mesoenatidae*

Epicleidium:	*Mesoenas variegata*	*Mesoenas unicolor*	*Monias benschi*
Depth	3 mm	3 mm	1.5 mm
Length	2.5 mm	2.5 mm	2.7 mm
Ligamentum Corpus claviculi:	broad and heavy	narrower and strong	thread-like or nearly absent

The body of the clavicle (*corpus claviculi*) is reduced or completely resorbed, although it remains as a strong ligament in *Mesoenas*—broader in *M. variegata* then in *M. unicolor*—and absent or so greatly reduced in *Monias benschi* as to make determination of its presence quite difficult. In *Monias*, it is reduced to a thin, thread-like cord which may be imbedded in the myofascia surrounding the coracoid muscles, and its identification as a *ligamentum corpus clavicula* is a matter of interpretation.

Dissection of a single specimen of *Apteryx australis mantelli* Bartlett (U.S.N.M.) revealed the complete absence of the *os claviculum*, nor was there satisfactory evidence to indicate a ligamentous vestige of this structure.

Glenny and Amadon (1954) found a similar reduction in the clavicles of *Otidiphaps nobilis* Gould, and, in addition, have listed several species of Barbets in which the furcula is incomplete except for a ligamentous pons between the separate clavicular elements, a condition similar to that found in a great many of the Old World Parrots. The amount of reduction in the body of the clavicle varies somewhat, but little exact information is readily available on this subject.

In Rand's account of the habits of the members of the Mesoenatidae (1936), he particularly stressed the point that these birds are typically terrestrial in habit. Flight appears to be absent in these birds and such escape attempts as were made were by foot and not by wing. In a more recent paper on the nests of *Mesoenas unicolor*, Rand (1951) pointed out that all of the three species of the Mesoenatidae are ground birds which either do not fly or fly poorly, and also stated that of two nests of *Mesoenas unicolor* which were found, both were located in the rain forest, and that they were located in a fork of a sloping tree and that in each case, the lower trunk of the tree had several low branches on it, thus making it possible for the bird to hop up to the nest.

Reduction in flight-function should not be construed to be directly correlated with and, in part, dependent upon the reduction in the clavicles, although instances of reduction or even deletion may be cited to substantiate this view (Kiwi, Ostrich, and other ratite birds).

In discussing the skeleton of the flightless Diatrymiformes, Lambrecht (1933) stated, "Claviculae verkümmert oder fehlend," and Matthew and Granger (1917) stated, that the clavical may be fused to the tip of the acromial process, in *Diatryma steini*, and if not, it is wholly absent as there is no facet for a separate bone.

Still other species of birds have partial or almost complete reduction of the clavicles and yet are capable of maintaining a satisfactory, at times even strong, flight-function. Examples of this are to be found among the Old World, and especially the Austral-asian, parrots, in which the epicleidium and a ligamentum corpus claviculi alone remains, and in *Otidiphaps nobilis* Gould, the Pheasant Pigeon, of New Guinea, which, although it inhabits the forest floor most of the time, still possesses the capacity for strong, powerful flight when occasion demands. On the other hand, *Rhynochetos jubatus*, the Kagu, of New Caledonia, has a well developed furcula, but its powers of flight are greatly reduced (Glenny, 1947) and escape is chiefly by running although this bird will fly for short distances when pursued. The pectoral muscles seem to be weak and poorly developed in this species, and this may account in part for its reduced flight capacity.

Other instances of flightless birds, in which the clavicles are present and fused to form a furcula, are found in *Atlantisia rogersi* (Lowe, 1928) and in *Notornis mantelli* (Parker, 1881). In both of these species, the typical gruiform furcula is present. In *Opisthocomus hoazin*, the clavicles, coracoids, and sternum are fused into a single complex of bone elements which affords rigid support, but which does not appear to contribute greatly to the flight-function since in this species, the capacity for flight is greatly reduced.

It should not be inferred that reduction or even loss of the clavicles results in a comparable reduction or loss of flight but that the latter may be due to a complex of factors, both structural and mechanical in nature along with certain less tangible ecological and behavioral factors. The Penguins and the Great Auk, *Pinguinis impennis* (Linn.), which lack the capacity for aerial flight, have the clavicle very well developed, even, in some cases, including a sizeable hypocleidium. These birds, however, are flightless only with regard to air, their mode of progression in the water is such as to merit saying that they fly in that medium instead of in the air above it.

It may be of interest to note that the Mesoenatidae, Raphidae, *Otidiphaps*, *Rhynochetos*, and *Apteryx*, to mention but a few examples, are or were insular species.

Geographical isolation may have contributed measurably to survival of forms with reduced capacity for flight. At the same time, however, a small, geographically isolated population, of any highly specialized form, is less apt to become highly variable, and thereby to develop any appreciable number of distinct species, through the evolution of multiple factors or characters, than is the case of a central or more generalized form, which has a large and widely distributed population,

wherein many lines of specialized evolution may develop. The Fringillidae may be cited as an example of this latter case. In such an instance, factors of competition need not be considered to be of such great importance so much as the effect of hybridization in large populations. Since most structural innovations tend to be recessive for a population, at least during the early period of occurrence, incidental specializations such as addition of structures, elaboration of existing structures, reduction and even loss of an existing structure are less apt to be perpetuated in a large, hybridizing population than in a smaller or a highly concentrated, isolated, inbreeding population.

In general, among the higher and more highly specialized vertebrates, there is a marked tendency towards reduction of previously existing (ontogenetic) structures than in the evolving of new elements.

Ultimately, success of either a large or small population depends upon its general capacity to survive under conditions of competition within the limits of the habitat. That survival factors have not seriously impaired the success of the Mesoenatidae, despite a holoterrestrial habitat, is evident. Contributing to their success may be the factors of geographical isolation, in Madagascar, and adoption of highly specialized ecological niches and a rather singular ecological stratification.

Both species of *Mesoenas* typically inhabit the forest floor, while *Monias* inhabits the low brush forest, in flat sandy country covered with dense brush (Rand, 1936). This may well be an important factor contributing to their success, since a good cover is afforded by the canopy above and fairly clear floor affords better visability and several directions for escape. As a result, flight is not to be construed as essential to survival in such a habitat, but survival may be facilitated by the ability to run and escape predators in this manner.

It is not clear how much pressure is brought to bear on these birds by natural predators. If this is not significant, then this becomes another factor which may have enhanced their opportunities for survival.

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A NONSURGICAL TREATMENT OF OPACITIES OF THE LENS AND OF THE CORNEA[1]

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For some time ophthalmologists have been seeking a method to treat opacities of the lens and of the cornea of the eye, commonly called cataracts and nebulae respectively. Surgical treatment has been somewhat successful and at the present time it is the principal method in use by the ophthalmologist.

As the Cataract Institute of South Kortright, New York, points out, the hazards associated with surgical removal of cataracts are numerous. They are important from both the physiological and economic standpoints. Since the incidence of cataracts is high, an increasingly urgent call is being placed upon researchers to develop non-surgical methods of effectively treating these opacities which obstruct the passage of light waves into the eye, ultimately causing varying degrees of blindness.

Experimental work was begun in 1952 with the purpose of making detailed studies of the effects of a specific plant extract on cataracts and nebulae of dogs and man.

The plant used for the treatment is *Sedum praealtum*. The method employed at present in treating an opacity is by direct application of the liquid content of the leaf of the plant.

Actual experimentation began in Guatemala City, Guatemala. With the help of Dr. Mariano Pacheco, former Secretary of Agriculture of the Republic of Guatemala, an abundant source of the plant was made available. With Dr. Pacheco's assistance the plant was keyed and confirmed as *Sedum praealtum* or "Santa Apolonia" as it is commonly called in Central America, its native habitat. Santa Apolonia is well known among the Indians of Guatemala for its "medicine juice" as they call it. Indeed, it was among these people that the application of the liquid content from the *Sedum* leaf to opacities of the eye, as well as inflammations occurring around the mouth, was first witnessed. Consequently, the first knowledge obtained about the properties of the plant was supplied by the very Indians who have used it as a medicine for many years. Much of their information seemed fundamentally superstitious. On the other hand, some of the facts learned from them were scientifically significant.

Since the author himself is a native of Guatemala, time and time again he has observed the crude Indian method of crushing the leaves of Santa Apolonia and applying the "juice" directly in the eye of one of their kind who bore an opacity of the eye or "pterigion" as they call it. Likewise, numerous times the author has personally observed the total recession of the opaqued tissues. It is important to bear in mind that there appears to be no tendency for the opacities to reappear after treatment with this substance the Indians call "eye medicine."

After careful examination of considerable evidence provided by the natives themselves and after an extensive study of the method employed by these people, a plan of treatment was formulated for applying this cure to eye opacities in dogs. Opacities of the lens and of the cornea were found to be quite common in Guatemala both in dogs and man.

The first case to which the treatment was applied was a three year old hound dog in which an outgrowth of tissue from the conjunctiva of the medial portion of the right eye covered approximately one-fourth of the surface of the cornea.

[1]This report represents a portion of the work done for a Master's thesis, under the supervision of Dr. Everett C. Myers, at Bowling Green State University.

The vision of the right eye up to this point was apparently not seriously impaired by the opaque tissue. The left eye was normal. The *Sedum* leaf-extract was placed directly on the eye surface, using 0.05 cc as a daily treatment. After the fourth day of treatment the abnormal growth began to recede with no irritation of the eye observable. After the eight day of treatment the opacity had almost completely disappeared from the surface of the cornea. After ten days, recession of the opacity was complete with no subsequent irritation effects noted. Visibility in the eye appeared to be essentially the same as before the treatment. Monthly observations have revealed no return of the growth, or any abnormality as a result of the treatment.

Another case similar to the one mentioned, and likewise of a dog, was treated by the same method. In this case, recession of the opacity of the cornea was complete after thirteen days of treatment.

The most interesting case which followed was that of a dog one year of age. The right eye contained an opacity which seemingly involved one-third of the lens. Lacking suitable instruments at the time prevented the exact localization or depth of this clouded condition. The opacity appeared as a thin white cataract originating from the medial angle of the eye but not sufficiently extended to obliterate vision in the eye. The left eye however, was closed and completely opaque with the nebula covering the entire surface of the cornea—by nebula meaning a cloudy opacity of the cornea. There was no visibility in the left eye.

The same kind of treatment previously employed was used in this case, applying 0.05 cc of the *Sedum* leaf-extract on the surface of each eye daily. There was ample "running" of the eyes—meaning an abundant release of tears, following the application of each treatment. After the fourth treatment, the left eye opened but obviously still with no visibility. After the ninth day recession of the opacity of the right eye was noticeable with some suggestion of the nebula of the left eye beginning to clear. The fourteenth day revealed the cataract of the right eye almost completely cleared. The left eye was partly cleared of the nebula which was receding both medially and laterally. At this stage however, recession of the nebula revealed an opacity which seemingly involved one-half of the lens in addition to the opacity of the cornea. Here again, lack of suitable instruments prevented the exact localization or depth of the opacity. Suggestions of visibility returning were observed in the left eye after the fourteenth day.

After the seventeenth day, treatment of the right eye was complete. The opacity had completely disappeared leaving no noticeable irritation of the eye. After the twenty-ninth day the opacities of both the cornea and the lens of the left eye had receded completely. A slightly pink color of the eye noticed at this time may have been due to slight irritation of the eye due to the treatment, however, visibility of the eye appeared normal. Monthly observations up to the seventh month show normal vision in both eyes and no evidence of re-occurrence of the opacities. No irritation effects have been noticed caused by the treatment except the momentary release of tears immediately following the application of the plant juice.

The cases mentioned dealing with treatment of dogs were followed by treatment of opacities found in three women.

The first of the three was a woman of Mayan Indian descent, forty-nine years of age, employed as a cook in a boarding house in Guatemala City. The right eye in this case appeared normal while the left eye contained a cataract, the opacity covering approximately one-third of the surface of the lens. Total possibility that this cataract did not involve the inner surface of the cornea in whole or in part rather than the lens could not be ascertained with absolute certainty. Visibility was not impaired by the opacity although the patient claimed the left eye bothered her when she worked more than ten hours a day in a kitchen with poor lighting.

The same treatment was applied to this patient; 0.05 cc. of the *Sedum* leaf-extract applied directly to the eye surface daily. After the first application, the patient claimed a slight burning sensation. There was an abundant release of tears from the eye after treatment was administered. After the eight treatment, the cataract showed evidence of beginning to recede. After the eleventh treatment the opacity appeared thinner and clearing. After the fifteenth day, more than one-half of the opacity had receded. The patient made no further complaints, apparently accustomed to the treatment. After the twenty-fourth treatment, the opacity seemed clear and treatment ceased two days later. The patient claimed the eye felt slightly irritated compared to the right eye. Monthy observations up to the sixth month has shown normal vision in the eye, no further irritation or other after effects and most important, no return of the cataract.

Two other cases followed the one just discussed. Both were in women over forty years of age. In both of these cases, the opacity was a nebula not covering the cornea enough to impair visibility. The standard treatment was employed with successful results in both cases. In one, fourteen treatments were necessary to clear the opacity while the other required seventeen. Both cases have been kept under observation and in neither has there been any irritable effects or re-occurrence of the opacity reported, the vision in both women remaining normal.

Other cases in dogs are undergoing treatment in Bowling Green State University at the present time and chemical analyses of the *Sedum* leaf-extract are in progress.

Recent information obtained from Guatemala City, indicates than an African plant of the same family as *Sedum* is suspected of also having the same property to treat such cases as the ones discussed. This plant and its properties is also under investigation at Bowling Green State University.

As was previously stated, much of the research on this problem is presently in progress with the major portion still to be undertaken. However, it is felt that the results obtained thus far are of sufficient significance to warrant submitting this preview report to inform interested persons of the research in progress.

SUMMARY

A nonsurgical treatment of opacities of the lens and of the cornea of the eye is reported. The method presently employed is by applying 0.05 cc of *Sedum praealtum* leaf-extract on the surface of the eye. Significant results are: a complete recession of the opacity, no serious subsequent irritation due to the treatment and most important, no observable tendency for the opacity to re-occur.

Soviet Science. *Ruth C. Christman*, Editor. American Association for the Advancement of Science (AAAS), Washington, D. C. 1952. vi+108 pp. $1.25.

A collection of papers presented at the symposium on Soviet Science sponsored by the AAAS Philadelphia in December, 1951. The fields of genetics, physiology and pathology, psychology and psychiatry, social sciences, soil science, physics and chemistry, and mathematics are discussed in some detail. In addition, consideration is given to the atomsphere under which Soviet scientists labor especially with respect to the scientist and intellectual freedom.

The contributors are individuals associated with American universities who are especially familiar with Soviet Science. In most cases, the authors are either of Russian ancestry or have had intimate contact with Russian science through study and travel. They view the Soviet sciences critically in comparison with "Western" science and try to give an honest appraisal of its strength and weakness.

In these days of tension when the relative progress of science and technology may well determine which way of life will dominate, this little book attempting to give an unbiased appraisal of the state of Soviet science is extremely timely and valuable.

Most parts of the book are well adapted to the information level of the general reader. However, of necessity there are technical details in some chapters which require a specialist in the field to fully appreciate.

EDWARD S. FOSTER, JR.

NEW RHYNCHOPHORA. I.

(COLEOPTERA, ANTHRIBIDAE AND CURCULIONIDAE)

ELBERT L. SLEEPER

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The following species were either collected by, given to or sent for identification to the author over a period of several years. This is the second of a series of papers to be published on new Rhynchophora.

LIST OF ABBREVIATIONS

DJ & JNK.....Dorothy J. and J. N. Knull.
ELS.....Elbert L. Sleeper.
(ELS).....E. L. Sleeper Collection.
JNK.....J. N. Knull.
(OSU).....Entomological Collection, The Ohio State University.

ANTHRIBIDAE

Ormiscus piercei nom. nov.

Ormiscus angulatus Pierce, 1930, p. 6.

I am suggesting the name *Ormiscus piercei* to replace *Ormicus angulatus* Pierce, the name *angulatus* being preoccupied by *Ormiscus angulatus* Jordan from Brazil.

Ischnocerus arizonicus n. sp.

Male: Elongate-subcylindrical, black, densely clothed with intermixed black and reddish-brown appressed setae, a small spot of white setae just in front of scutellum, prothorax and elytra tuberculate.

Rostrum two thirds as long as pronotum; spatulate, flattened, densely, coarsely, shallowly punctate; with a fine median carina and two large lateral carinae; middle depressed from between eyes to apical third; sparsely clothed with reddish-brown setae. Antennae attaining apical two-thirds of prothorax, reddish-brown. Scrobes small, covered, with carina above.

Head densely, finely punctate, densely clothed with recumbent, reddish-brown setae. Eyes lateral, elliptical, coarsely granulated.

Prothorax narrowing apically from base, sides angulate; pronotum with a median and two lateral tubercles, two divergent raised areas in apical third meeting at middle of apex; densely clothed with black and reddish-brown setae, basally a densely pubescent white spot at middle. Carina prebasal, strongly sinuate, obtusely angulate at middle, bent abruptly forward at sides terminating in a prominet tubercle. Scutellum small, prominent, pointed.

Elytra elongate, sides parallel in basal three-fourths; densely clothed with black and reddish-brown setae; base strongly sinuate; humeri prominent, rounded; disc multi-tuberculate; strial punctures small, fine, shallow, distant; intervals one without tubercles, second intervals with a moderately large pointed tubercle in basal fourth and many small tubercles, third intervals without tubercles, fourth intervals with a very large tubercle occupying entire basal fourth of intervals, with prominent tubercles at middle and at declevity, remaining intervals with small tubercles.

Ventral side strongly convex; prosternum densely, coarsely punctate; ventral abdominal segments 1-4 with minute, distant punctures, fifth densely, deeply punctate. Femora and tibiae unarmed. First tarsal segment as long as second. Claws large, divergent, minutely toothed within.

Length 9.3 mm., width 4.1 mm.

Type Locality.-Unique holotype, male, Huachuca Mts., Ariz. VII-15, C. W. Jones in (ELS).

Wholly unlike any other North American species in this genus or is it closely related to any of the members from Central and South America. It appears to be nearest to *I. aenus* Jordan from Brazil and Paraguay, but differs in the arrangement of the tubercles on the elytra.

Stenocerus knullorum Sleeper

Stenocerus knullorum Sleeper, 1953, p. 114

Through an oversight a paragraph was left out of the original description of this anthribid. It is as follows: "In general appearance this resembles *S. platalea* Jordan, from which it may be separated by the shorter prosternal lamella of the latter. Nearest *longulus* Jekel, but does not have the mesosternal process bituberculate or the prosternal lamella notched between coxae.

Gymnognathus chiricahuae n. sp.

Figure 1.

Female: Elongate, subcylindrical; black with red humeral area; vestiture black and gray, the gray condensed in spots on prothorax and elytra.

Rostrum spatulate, flat and broad; three-fourths as long as pronotum; with a raised keel-like carina basally, coarsely, closely punctate throughout, punctures of basal two-thirds obscured by gray pubescence. Antennae inserted on sides of rostrum, reaching posterior margin of prothorax in female, (to middle of elytra in male), segments clavate. Club loose, densely clothed with short, fine, black pubescence.

Head confluently punctate forming strigae, not foveate between eyes. Eyes lateral, rounded.

Prothorax narrowing apically; sides straight; carina feebly arcuate, prebasal, extending forward laterally in basal half. Pronotum black, sides of prothorax and base covered with dense gray pubescence.

Elytra as wide as prothorax; sides parallel; base strongly sinuate; disc strongly flattened; humeral area bright red extending backward in basal third; disc and apex of elytra with short, appressed, gray setae; punctures of striae fine, deep, separated by about their own diameter; intervals flat, densely, confusedly, minutely punctate. Pygidium exposed, vertical, clothed with dense appressed gray setae.

Ventral side strongly convex; densely covered with appressed gray setae. Legs clothed with appressed gray pubescence. Femora robust. Tibiae straight, thin. First segment of tarsi as long as remaining segments combined. Claws large, divergent, simple.

Length 5.6 mm., width 2.5 mm.

Type Locality.-Holotype, female, Onion Saddle, Chiricahua Mts., Ariz. VIII-2-52, DJ & JNK. in (OSU). Allotype same data in (OSU). Fifteen paratypes; 1 same data as types in (ELS); 6 same data in (OSU); 2 Chiricahua Mts., Ariz. VII-26-52, DJ & JNK. in (OSU); 1 same data in (ELS); 2 Chiricahua Mts., Ariz. VIII-12-52, DJ & JNK. in (OSU); 2 same data in (ELS); 1 Chiricahua Mts., Ariz. VIII-19-52, DJ & JNK. in (OSU).

Host.—Beaten by DJ & JNK. from oak (*Quercus* sp.) at approximately 8000 feet in the Chiricahua Mts.

Not closely related to any other members of the genus, all of which are from tropical America. Nearest *G. abundans* Jordan to which it keys (Jordan, 1906). It differs from *abundans* in that the latter has the pronotum at middle clothed with white pubescence, the lateral areas are reddish. *G. abundans* does not have the red humeral areas.

CURCULIONIDAE

Erethistes arizonicus n. sp

Figure 5.

Male: Elliptical, black, shining, sparsely clothed with recumbent, curved, broad, white scales.

Rostrum one third longer than pronotum, feebly curved, cylindrical; impunctate median line in basal half; sparsely, finely punctate throughout, denser laterally in base. Antennae inserted beyond middle, dark reddish-brown. Funicle 7-segmented, first and second segment elongate, first twice as long as second, remaining segments round, as long as broad. Club ovate, densely clothed with fine, short, brown pubescence.

Head with close, moderately coarse punctures, an elongate fovea between eyes. Eyes feebly convex, finely granulate.

Prothorax broader than long, feebly convex, sides arcuate; with irregular, closely placed punctures each with a broad, recumbent, white scale; interspaces smooth, shining. Scutellum round, denuded, coarsely punctate.

Elytra sub-triangular, sides strongly convergent to apex; humeri not prominent; striae narrowly, feebly impressed, punctures coarse, deep, not setiferous; intervals raised as are the interspaces between the strial punctures; sparsely clothed with broad white scales arranged in groups giving elytra a mottled appearance; each strial interspace with a narrow, recumbent, white scale.

Ventral side densely clothed with recumbent, broad, white scales; finely punctate. Legs elongate, sparsely clothed with narrower, white scales; densely punctate. Femora dentate mesially; hind femora extending beyond apex of abdomen. Tibae mucronate at inner apical angle, with a short claw at outer angle.

Length 6.3 mm., width 2.8 mm.

EXPLANATION OF FIGURES IN PLATE

1. *Gymnognathus chiricahuae* n. sp., ♂ (allotype).
2. *Myrmex subglaber* (Schaeffer).
3. Prothorax of *Myrmex horni* n. sp., ♀.
4. Prothorax of *Myrmex chisosensis* n. sp., ♀.
5. Apex of anterior tibiae of *Erethistes arizonicus* n. sp., ♂.
6. Anterior femur of *Myrmex horni* n. sp., ♀.
7. Anterior femur of *Myrmex chisoensis* n. sp., ♀.

(Line = 1 mm.)

Type Locality.—Unique holotype, male, Madera Canyon, Santa Rita Mts., Ariz., VII-15, C. W. Jones Collr. (ELS).

Resembles *E. truncatus* Champion from Honduras, but is more elongate, narrowed and has the tibiae with a short claw at outer apical angle. Because of the latter characteristic *arizonicus* must be placed close to *E. cyanipes* Champion from Ventanas, Durango, Mexico, which the former differs from in smaller size, abscence of "aeneous or bluish lustre", round instead of oval scutellum and prosternum not emarginate in front. The genus *Erethistes* Pascoe should be placed after *Cholus* Germ in the Leng Catalogue.

Thysanocnemis arizonicus n. sp.

Female: Oblong, feebly convex, brownish-yellow, rostrum and antennae reddish-brown; moderately clothed with depressed, narrow, yellowish-white scales; two dark V-shaped fascia behind middle and at declivity of elytra; elytral intervals subequal in width.

Rostrum one-half longer than pronotum, strongly curved, slender, cylindrical, smoother at middle, minutely punctate laterally. Scrobes deep, directed against eye. Antennae inserted at middle; scape attaining eye; first segment of funicle as long as following three combined, second as long as following two. Club very elongate and narrow, first two segments of club composing three-fourths of club; densely clothed with short, fine pubescence.

Head closely, finely punctate, sparsely clothed with narrow, depressed, yellowish-white scales. Eyes separated by less than one-half their diameter. Eyes coarsely granulate.

Prothorax broader than long, feebly convex, sides feebly arcuate; apex feebly constricted; finely, densely punctate, puctation almost obsured by dense, narrow, yellowish-white scales. Scutellum triangular, densely clothed with broad white scales.

Elytra oval, sides parallel in basal half, thence converging to apex; striae feebly impressed, strial punctures small, round deep, about their own diameter apart, each bearing a narrow white scale; intervals minutely punctate, moderately clothed with narrow yellowish-white scales; dark fascia composed of light brown scales.

Ventral side moderately clothed with spatulate, white scales, sparser on abdominal segments. Abdominal segments sparsely, finely punctate; first suture straight, second angulate at sides; fifth segment as long at middle as preceding two. Legs pale yellowish-brown, stout, sparsely clothed with white hair-like scales. Femora greatly thickened, unarmed. Anterior tibiae straight, feebly flattened, feebly arcuate mesially. Tarsal claws with long, convergent teeth.

In male rostrum shorter, first segment of funicle shorter, more robust; fifth abdominal segment shallowly impressed at middle.

Length 2.6 mm., width 1.5 mm.

Type Locality.—Holotype female, Chiricahua Mts., Ariz., VII-20, C. W. Jones Collr. in (ELS). Allotype and two paratypes same data in (ELS).

Keys to *T. squamiger* Casey, but *squamiger* differs as follows: rostrum shorter, thicker; scales of elytra shorter, broader; elytra without fascia; fifth abdominal segment not impressed at middle and femora not as robust.

Thysanocnemis yavapaiensis n. sp.

Male: Elongate, black, densely clothed with yellow, brown, black, white, and gray appressed, narrow scales, and erect, short, robust, black and white scales. The white scales forming three transverse fascia on elytra.

Rostrum as long as pronotum; with smooth, median carina; finely, seriately punctate forming lateral carina between series; sparsely clothed basally with narrow, brown and white scales. Antennae reddish-brown; inserted in apical third; first segment of funicle longer than second, second as long as third and fourth combined. Club elongate-oval, black densely, clothed with fine pubescence.

Head densely clothed with brown and white narrow scales obscuring fine, dense punctures. Eyes separated by about one-fourth their width, not prominent, coarsely granulated.

Prothorax broader than long, slightly convex; sides subparallel in basal two-thirds, narrowing suddenly to a faint apical constriction; densely clothed with transversely-arranged brown and yellow, narrow scales, with a narrow, median white line of narrow scales in basal two-thirds; vestiture obscuring fine, dense punctures. Scutellum densely clothed with broad white scales.

Elytra elongate, one-third wider than prothorax; tuberculate at declevity, sides subparallel, converging to apex; humeri oblique, prominent with a distant patch of yellow and brown scales; striae faintly impressed, punctures small, shallow, setiferous; intervals flat bearing depressed, narrow scales and short, robust, erect scales; first interval at middle of disc with a dense patch of yellow and brown narrow, depressed and short, robust, erect scales; third interval with a crest of black, erect, broad scales at middle of disc; vestiture of elytra dense obscuring punctures, composed of intermixed black and gray, narrow, depressed scales interrupted by three fascia of white scales, first immediately behind humeri, second at middle of elytra in front of declivity, third at apex. Tubercles of elytral declevity with a large tuft of broad, black scales.

Ventral side densely clothed with narrow, white and brown scales obscuring sparse shallow punctures of abdominal segments. First abdominal suture straight, others strongly angulate on sides. Pygidium partly exposed, vertical, densely clothed with brown, white and yellow, narrow, erect scales. Legs stout, densely clothed with narrow, white recumbent scales. Femora reddish-brown in proximal and distal fourths, middle black. Tibiae reddish-brown, slender, with a ring of dark scales at middle and a short spine at tip. Tarsi reddish-brown. Claws with long, slender, convergent teeth.

Length 3.4 mm., width 1.7 mm.

Type Locality.—Holotype, male, Ironsprings, Ariz., VI-22-37, in (ELS). One paratype same data in (ELS).

Closely related to *T. graphica* Casey, which differs as follows: form narrower, less elongate; broader scales of vestiture of prothorax and elytra; tibiae without dark ring of scales; tooth of claws shorter, stouter; elytral tubercles smaller.

Neotylopterous baboquivariensis n. sp.

Male: Elongate, black, rostrum, antennae and tibiae reddish-brown; densely clothed with intermixed light and dark gray, narrow scales with semierect, spatulate scales. Head, prothorax and humeri densely clothed with yellow, white and reddish-brown, narrow, depressed scales. Apical third of each elytron with three tufts of white and black scales.

Rostrum as long as pronotum, with smooth median line, finely, deeply punctate laterally; sparsely clothed with narrow, white scales. Scrobes deep, directed against eyes. Antennae inserted in apical third, scape attaining eyes; first segment of funicle longer than second, second longer than third and fourth combined; segments of funicle with long, white bristles. Club very elongate, densely pubescent.

Head densely clothed with white and brown scales obscuring fine, dense punctation. Eyes coarsely granulate, separated by less than one-tenth diameter of eyes.

Prothorax convex, sides arcuate, narrowing to apical constriction at apex; densely clothed with reddish-brown, yellow and white scales which are transversely arranged obscuring fine punctures of pronotum. Reddish-brown scales condensed into spots at middle of pronotum, separated by a median white line, Scutellum triangular, densely clothed with broad white scales.

Elytra elongate, one half wider than prothorax, sides subparallel in basal half then convergent to apex; striae not impressed, punctures round, shallow, distant; intervals densely, minutely punctate; vestiture dense, obscuring punctures, clothed with intermixed, gray and black, narrow scales and semierect, spatulate, gray and black scales; humeri densely clothed with yellow, white reddish-brown scales, a dense patch of dark reddish scales each side of scutellum; apex of each elytron with three prominent tufts of black and white, spatulate scales.

Ventral side densely clothed with spatulate, white and gray scales with intermixed semierect, white hairs; abdominal segments finely, sparsely punctate; first abdominal suture straight at middle, angulate at side; second suture strongly angulate at sides, second segment attaining fourth, fifth abdominal segment longer at middle than preceding three segments. Femora densely clothed with flat, broad scales and long, bristly, white hairs, denser mesially, unarmed. Anterior tibiae slender, bent, sparsely clothed with narrow, white scales; densely clothed mesially with long white hairs; feebly spined at tip. Tarsal claws large, divergent, strongly toothed.

Length 4.0 mm., width 2.1 mm.

Type Locality.—Unique holotype, male, Presumido Pass, Baboquivari Mts., Ariz., VII-12, in (ELS).

This probably belongs in a new genus, but for the present I am placing it in *Neotylopterous* Hustache because of the structure of the second abdominal segment and the form of the depressed vestiture. Not allied to any other member of the genus. Nearer *N. pallidus* (LeConte) than any other species in the genus. Differs from *pallidus* in form, arrangement of vestiture, yellow humeral areas, tubercles on apex of elytra, form and vestiture of the anterior tibiae.

Myrmex horni n. sp.

Figure 3 and 6.

Female: Moderately elongate, shining, black, antennae and tarsi dark reddish-brown; clothed with short, semierect, white setae and longer, erect black setae; white setae on elytra condensed in a line on intervals 2, 4, 6, 8, 9, and 10, black setae condensed in a single straight line on intervals 1, 3, 5, and 7; pronotum not carinate throughout.

Rostrum shorter than pronotum, deeply sulcate along smooth median line, coarsely, deeply punctate laterally in base. Antennae inserted in apical third, scape exceeding anterior margin of eyes; first segment elongate, remainder rounded, subequal in length. Club prominently four-segmented, ovate, densely clothed with short, fine pubescence.

Head closely, coarsely punctate, a shallow depression on vertex; sparsely clothed with recumbent white and erect black setae. Eyes strongly convex, separated by more than half diameter of eye.

Prothorax longer than broad, not noticeably constricted at base, sides arcuate; without smooth impunctate areas; contiguously, coarsely, deeply punctate; sparsely clothed with recumbent white setae and long erect black setae; vestiture on prothorax directed forward. Scutellum small, densely clothed with white setae.

Elytra elongate, feebly convex, one-third wider than prothorax; humeri prominent; striae feebly impressed, strial punctures deep, round, less than their own diameter apart; intervals feebly convex, intervals 1, 3, 5, and 7, with minute, distant punctures in a single line, each with a long thin, erect, black setae; intervals 2, 4, 6, 8, 9, and 10 with minute, close punctures confusedly placed, each with a semierect white setae.

Ventral side moderately clothed with long, fine white hairs which are denser on sides; abdominal segments minutely, sparsely punctate. Legs sparsely punctate, moderately clothed with intermixed, long, erect, black and white setae. Femora armed with a very small, blunt, nearly obsolete tooth. Anterior tibiae flattened, sinuate mesially, widest part in proximal fourth. Tarsal claws large, divergent, simple.

Length 4.6 mm., width 1.5 mm.

Type Locality.—Holotype female, Morris Ranch (Gillespie Co.), Texas, V-7, in (ELS). One paratype, same data, in (ELS).

Unlike any other known species from the United States, (except the following species). Nearest *M. hystricosa* (Champion) from Mexico, which is larger, has the pronotum much more finely and sparsely punctate, especially in the basal half; the white setae on elytra are broader and more recumbent. *M. horni* is remarkable in having the femoral tooth much smaller than any known species.

Named for Dr. G. H. Horn, one of the greatest American Coleopterists.

Myrmex chisosensis n. sp.

Figure 4 and 7.

Female: Moderately elongate, shining, black, antennae and tarsi dark reddish-brown; clothed with short, semierect, white setae and longer, erect, black setae; white setae on elytra condensed in a line on intervals 2, 4, 6, 8, 9, and 10, black setae condensed in a single straight line on intervals 1, 3, 5, 7; pronotum strongly carinate throughout.

Rostrum much, shorter than pronotum, smooth along median line, coarsely, confluently punctate laterally in basal half, finely punctate apically; a short, deep sulcus each side of median line in basal third. Antennae inserted beyond middle, scape attaining middle of eye, funicle seven-segmented; first segment elongate, one-fourth longer than second, remainder elongate, cylindrical, subequal in length. Club prominently four-segmented, ovate, finely, densely pubescent.

Head strongly convex, coarsely punctate, over their own diameter apart, a small, deep, elongate puncture on vertex; very sparsely clothed with prostrate white hairs and erect black hairs. Eyes strongly convex, separated by over half their diameter.

Prothorax longer than broad, not tubulate, feebly constricted at base, sides evenly arcuate; with smooth, impunctate median carina; coarsely, irregularly punctate, with smooth impunctate areas between punctures; sparsely clothed with semierect white setae and long erect, black setae. Scutellum densely clothed with white scales.

Elytra elongate, one-half wider than prothorax, sides subparallel in basal half; striae very feebly impressed, strial punctures round, coarse, deep, separated by half their diameter; intervals nearly flat, punctures of intervals fine, intervals two confusedly punctate, remainder with punctures in a single straight line.

Ventral side sparsely clothed with long, fine hairs, on abdomen a few radiate-pectinate scales intermixed with long white hairs, abdominal segments finely, sparsely punctate; fifth segment longer than third and fourth combined, feebly emarginate at tip. Legs finely, sparsely punctate, sparsely clothed with long, erect, black and white hairs. Femora armed with a small, narrow, sharp tooth mesially. Anterior tibiae flattened, strongly sinuate mesially, broadest in proximal third. Tarsal claws divergent, simple.

Length 4.0 mm., width 1.5 mm.

Type Locality.-Unique holotype, female, Mariscal (Brewster Co.), Texas VII-15 in (ELS).

Nearest *M. horni n. sp.*, but the latter has the femora armed with a very small, blunt, nearly obsolete tooth, the intervals of the elytra more convex, the strial punctures more deeply impressed, and the pronotum not carinate more densely, closely, punctate.

Myrmex knulli n. sp.

Male: Elongate, shining, black, antennae and tarsi dark reddish-brown; sparsely clothed with long, fine, erect, intermixed black and white hairs.

Rostrum shorter than pronotum, straight, smooth along middle, coarsely punctate-sulcate in basal half, finer apically; with sparse long, erect, black hairs. Antennae inserted beyond middle, scape attaining anterior margin of eyes; funicle seven-segmented, first segment twice as long as second, remainder subequal in length. Club ovate, densely clothed with short, fine brownish pubescence.

Head convex, coarsely, densely, deeply punctate; coarsely punctate between eyes; sparsely clothed with long, erect, black hairs. Eyes convex, separated by more than three-fourths their own diameter.

Prothorax longer than broad; sides rounded; strongly narrowed in basal fourth; coarsely, closely punctate throughout, sparsely clothed with intermixed, erect, long white and black hairs. Scutellum densely clothed with white pubescence.

Elytra twice as wide as prothorax, sides subparallel; striae feebly impressed, strial punctures impressed, deep, coarse, round, close-set, about half their diameter apart; intervals flat, punctures of intervals fine, distant, irregularly placed.

Ventral side sparsely clothed with fine, long, erect, white hairs; abdominal segments finely, densely punctate, fifth segment shorter than third and fourth combined. Legs clothed with long, erect, intermixed, black and white hairs. Femora armed with a small, narrow, sharp tooth. Anterior tibiae feebly sinuate mesially, broadest part before middle.

Length 4.9 mm., width 2.0 mm.

Type Locality.-Holotype, male, Davis Mts., Texas VII-2-40 DJ & JNK., in (OSU). Allotype, same data, in (OSU). Fifteen paratypes: 3 same data as types in (OSU); 2 same data in (ELS); 8 Jeff Davis Co., Texas VI-20-52, DJ & JNK., in (OSU); 2 same data in (ELS).

Close to *M. corae* (Champion) from El Cora in Tepic, Mexico. The types of *knulli* have been compared with the type of *corae* by G. A. K. Marshall. It differs from *corae* in that *knulli* has the white setae longer and not recumbent, and the front between the eyes coarsely, densely punctured instead of smooth. This has previously been identified as *M. corae* by some American authors.

Named for J. N. Knull in recognition of his kind help to the author in his studies of the Curculionidae.

Myrmex knulli chiricahuae n. sp.

Otidocephalus corae Champion, Schaeffer, 1907, p. 77 (nec Champion 1903).

Female: Form, color and vestiture as in *Myrmex knulli knulli*, differs as follows: pronotal punctures coarser, deeper and denser; elytral intervals convex, strial punctures deeper; abdominal segments more coarsely, densely punctate; anterior tibiae more strongly sinuate.

Length 4.6 mm., width 1.9 mm.

Type Locality.-Holotype, female, Chiricahua Mts., Ariz. VI-27-49, DJ & JNK. in (OSU). Allotype same data in (OSU). Forty paratypes: 1 Huachuca Mts., Ariz. VI-9-32 JNK., 3 Chiricahua Mts., Ariz. VII-14-36, JNK., 2 Huachuca Mts., Ariz. VII-20-36, JNK., 1 Huachuca Mts., Ariz. VII-20-37. DJ & JNK., 1 Chiricahua Mts., Ariz. VII-20-37 DJ & JNK., 2 Chiricahua Mts., Ariz. VII-19-52, DJ & JNK., 16 Chiricahua Mts., Ariz. VII-26-52, DJ & JNK., 6 Chiricahua Mts., Ariz. VIII-2-52, DJ & JNK., 2 Chiricahua Mts., Ariz. VIII-12-52, DJ & JNK., in (OSU); 3 Huachuca Mts., Ariz. VII-20-37, DJ & JNK., 2 Chiricahua Mts., Ariz. VII-26-52, DJ & JNK., 1 Chiricahua Mts., Ariz. VI-27-49, DJ & JNK. in (ELS).

This is the *Otidocephalus corae* of Schaeffer. His determinations were made from the original description only. Examples were sent to the British Museum for comparison with type and were found to be unlike *corae*, *chiricahuae* differing in the coarsely punctured front between the eyes, convex elytral intervals, and erect white pubescence. From *arizonica* it may be separated by the small femoral tooth.

Myrmex schaefferi n. sp.

Female: Elongate-oval, shining, black, moderately sparsely clothed with narrow scale-like white setae, intervals of elytra with short, robust black hairs.

Rostrum slightly shorter than pronotum, robust, stout, smooth along middle, densely, strongly punctate at sides, sparsely clothed with short, black setae and robust white scale-like setae. Antennae black, funicle segments 2-6 as long as broad, second and third segment equal. Club elongate oval, densely, finely pubescent, as long as preceding three segments.

Head smooth, shining, with coarse punctures; a very deep fovae on vertex; densely, coarsely punctured between eyes. Eyes widely separated.

Prothorax obconical, apical margin sinuate at middle; densely, coarsely punctate with some smooth areas, and impunctate median line; sparsely clothed with robust white setae. Scutellum triangular, densely pubescent.

Elytra elongate-oval, widest behind middle, about twice as wide as prothorax; humeri obliquely truncate; moderately clothed with short, narrow, scale-like, white setae and short robust black hairs; striae with deep, coarse punctures which are deeply impressed in basal third of sutural striae; intervals flat, with minute, setiferous punctures, irregularly placed.

Ventral side and legs moderately clothed with white setae as on dorsum; abdominal segments densely, coarsely punctate. Femora elongate, finely punctate; mesial tooth small, triangular, blunt. Anterior tibiae elongate, very slender, nearly straight within.

Length 4.5 mm., width 2.0 mm.

Type Locality.-Holotype, female, Fort Bowie, Ariz. VII-25, in (ELS). Allotype, same data, in (ELS). Four paratypes: 2 Fort Bowie, Ariz. VII-25, 1 Fort Bowie, Ariz. VIII-10, in (ELS); 1 Fort Bowie, Ariz. VII-25, in (OSU). All the examples of this weevil were probably collected in the Chiricahua Mts., behind Fort Bowie.

Superficially resembles *M. estriata* (Casey), but can be easily separated by the elytral punctures and scale-like setae. Nearest *M. suglaber* (Schaeffer), however, the latter has the intervals convex, strial punctures very deeply, strongly impressed. *M. subglaber* is shorter, more robust and with sparser vestiture.

Myrmex subglaber (Schaeffer)

Figure 2.

Otidocephalus subglaber Schaeffer, 1907, p. 76.

A single example of this rare little weevil has been sent to the author. On it is the following data: "Santa Rita Mts., Ariz. VII-11" in (ELS). Two examples are known other than the type

in the Schaeffer Collection at Brigham Young University, Provo, Utah from "Arizona." They are in (OSU) bearing the following data: "Palmerlee, Ariz. H. A. Kaeber" and "Huachuca Mts., VII-20-37, DJ & JNK."

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Fundamentals of Biology. *M. J. Harbaugh* and *A. L. Goodrich*, Editors. The Blakiston Company, Inc., New York. 1953 x+611 pp. $6.00.

This text consistently fulfills its purpose of setting forth fundamentals of biology. Many so-called general biology texts begin with a biological approach, but after more or less of preliminary statement settle down to a study of plants and animals as separate entities. In each of the categories of this book, which are roughly: 1) physical basis of life in its environment (chapters 1–4), 2) fundamental life processes (chapters 5–14), 3) heredity (chapter 15), 4) evolution (chapter 16), and 5) ecology (chapters 17–20), the editors achieve a well-balanced treatment of life in its diversified forms. This is somewhat remarkable in view of the fact that both Harbaugh and Goodrich are zoologists. Another zoologist, an entomologist, a bacteriologist and a botanist comprise the rest of the contributing staff. In addition to the integration of living forms, the relationship of other organisms to man is adequately treated.

The book is well illustrated with pictures and diagrams, and, where feasible, tables and outlines are effectively employed. The text is designed for a "two-semester, eight-credit hour course covering the general field of biological sciences", and its use should prove to be a rewarding experience for the teacher as well as for the student.

E. Eloise Whitwer.

Excerpta Medica-Cancer. *R. van Dam* and *W. van Westering*, Editors. Excerpta Medica, N. V., Amsterdam, The Netherlands. 12 Nos. yearly $10.00.

The Excerpta Medica consists of 16 abstracting journals all begun since the war; of these the one devoted to cancer is the most recent. Three other journals in this series of interest to biologists as well as physicians deal with anatomy, physiology and biochemistry, and endocrinology. The cancer journal presents its subject matter under 15 headings among which are experimental research, plant and animal tumors, the various organ systems (8), diagnosis, treatment, statistics, and cancer control. The abstracts in this first number (July 1953) are generally adequate and deal with reasonably recent articles; most appeared in 1952.

H. Schlumberger.

PARASITES OF SOME OHIO AMPHIBIA[1,2]

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Reports of parasites from amphibian hosts in Ohio are few in number. There are two published reports each dealing with but a single species of parasite or with a single group. Stunkard and Cable (1931) described the copepod *Lernaea ranae* from tadpoles of *Rana clamitans* taken near Cincinnati; Hazard (1937, unpublished in part) examined approximately 850 frogs and toads from Clinton County and reported 5 species and 2 subspecies of *Opalina* from these hosts. Other parasites observed were *Zelleriella intermedia cuneata*, *Nyctotherus cordiformis*, *Haptophrya michiganensis*, and *Trichomonas augusta*. In addition, Hazard reported *Euglenamorpha hegneri* and *Entameba ranarum* from about 500 *Rana clamitans* tadpoles; Groves (1945), studying the ecology of *Desmognathus fuscus* in Licking County, reported *Phyllodistomum solidum* from the urinary bladder of these salamanders; Ritter (1950) described *Opalina obtrigonoidea* and *Nyctotherus cordiformis*, among others, from *Rana pipiens* collected in Hocking, Hardin, Franklin, and Fairfield Counties.

During the summer and fall of 1952, amphibia were collected from 19 stations in 7 Ohio counties, with the majority of the hosts being taken in Franklin and Hocking Counties. The frogs were from Ottawa, Erie, Logan, and Trumbull Counties, whereas the majority of the salamanders were taken from Hocking County. Specific stations by county are listed in table 1.

Following is a list of the hosts collected and parasites found:

Acris crepitans

The only parasite found was the ciliate *Opalina obtrigonoidea* in the large intestine.

Bufo americanus

The protozoan *Opalina obtrigonoidea* in the large intestine; the cestode *Distoichometra bufonis* in the small intestine.

Desmognathus fuscus

The trematodes *Brachycoelium salamandrae* and *Plagitura salamandra* from the small intestine; the cestode *Crepidobothrium amphiumae* from the small intestine; the nematode *Cosmocercoides variabilis* from the large intestine; numerous acanthocaphalan cysts attached to the mesenteries of the body cavity. No phyllodistomes were recovered from the urinary bladder, lending weight to Grove's (1945) statement that "*Phyllodistomum solidum* is not a common parasite of *Desmognathus fuscus*."

Eurycea longicauda

No parasites were found in these salamanders.

Hyla crucifer

The trematode *Glypthelmins quieta* from the small intestine; the nematode *Oswaldocruzia pipiens* from the small intestine.

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[1]This work was done while the author was a Muellhaupt Fellow in Biology in the Department of Zoology and Entomology, Ohio State University, during 1952–1953.

[2]Published with the aid of a grant to The Ohio Journal of Science from the Charles F. Kettering Foundation.

Necturus maculosus

The cestode *Crepidobothrium lönnbergi* from the small intestine.

Plethodon cinereus

No parasites were recovered from these salamanders.

Pseudacris triseriata

The protozoan *Opalina obtrigonoidea* from the large intestine; the trematode *Brachycoelium salamandrae* from the small intestine; the nematode *Cosmocercoides variabilis* from the large intestine.

Rana catesbeiana

Opalina obtrigonoidea from the large intestine; *Glypthelmins quieta* from the small intestine.

TABLE 1

Stations by county where amphibia were collected

Ottawa	Fairfield	Franklin	Hocking	Logan	Erie	Trumbull
McGee Marsh	Canals near Sugar Grove	Indian Run	Conkle's Hollow	Mac-o-chee Creek	Marsh on Sandusky Bay	Mosquito Creek near Warren
Portage River	Cranberry Bog, Buckeye Lake	Hayden Run	Crane Hollow			
		Walnut Creek				
		Scioto River	Venard's Run			
		Olentangy River	Springs near Bremen			
		University Pond	Springs near Dr. Thomas' Cabin			

Rana clamitans

Nyctotherus cordiformis from the large intestine; *Gorgoderina tanneri* from the urinary bladder, the first report of this parasite from *Rana clamitans; Cosmocercoides dukae* from the large intestine; cysticerci and encysted acanthocephalan larvae from the mesenteries of the body cavity.

Rana pipiens

Opalina obtrigonoidea and *Nyctotherus cordiformis* from the large intestine; *Gorgoderina attenuata* from the urinary bladder; *Haematoloechus complexus* from the lungs; *Megalodiscus temperatus* from the large intestine; metacercariae of *Apharyngostrigea pipientis* (*Tetracotyle pipientis*) encysted in masses around the urinary bladder, heart, and on the muscles of the throat; *Rhabdias ranae* from the lungs; *Oswaldocruzia pipiens* from the small intestine; *Cosmocercoides variabilis* from the large intestine; cysticerci from mesenteries of the body cavity.

Rana sylvatica

Opalina obtrigonoidea from the large intestine; *Brachycoelium salamandrae* from the small intestine, constituting a new host record; *Oxysomatium americanum* from the large intestine; larval acanthocephala encysted on the mesenteries of the body cavity.

SUMMARY OF PARASITES REPORTED FROM OHIO AMPHIBIA

(Data from Stunkard and Cable (1931), Hazard (1937), Groves (1945), Ritter (1950), Odlaug (present paper)

Acris gryllus: *Opalina virguloidea magninucleata, Nyctotherus cordiformis, Trichomonas augusta.*

Acris crepitans: *Opalina obtrigonoidea, Nyctotherus cordiformis, Trichomonas augusta.*

Bufo americanus: *Opalina obtrigonoidea americana, Nyctotherus cordiformis, Trichomonas augusta, Distoichometra bufonis.*

Desmognathus fuscus: *Plagitura salamandra, Brachycoelium salamandrae, Crepidobothrium amphiumae, Phyllodistomum solidum,* larval acanthocephala.

Eurycea bislineata: *Phyllodistomum solidum.*

Hyla crucifer: *Opalina pickeringi, Nyctotherus cordiformis, Trichomonas augusta, Glyphthelmins quieta.*

Hyla versicolor: *Opalina hylaxena, Nyctotherus cordiformis, Trichomonas augusta.*

Pseudacris triseriata: *Opalina chorophili, Nyctotherus cordiformis, Trichomonas augusta, Brachycoelium salamandrae, Cosmocercoides variabilis.*

Necturus maculosus: *Crepidobothrium lönnbergi.*

Rana catesbeiana: *Glypthelmins quieta.*

Rana clamitans: *Nyctotherus cordiformis, Trichomonas augusta, Megalodiscus temperatus, Gorgoderina tanneri,* cysticerci.

Rana clamitans tadpoles: *Opalina larvarum, Euglenamorpha hegneri, Entameba ranarum, Lernaea ranae.*

Rana pipiens: *Opalina obtrigonoidea, Nyctotherus cordiformis, Trichomonas augusta, Trichomonas batrachorum, Trichomitus parvus, Hexamitus intestinalis, Chilomastix caulleryi, Trepomonas agilis, Retortomonas dobelli, Entameba ranarum, Megalodiscus temperatus, Haematoloechus complexus, Gorgoderina attenuata, Cosmocercoides variabilis, Rhabdias ranae, Oswaldocruzia pipiens,* metacercaria of *Apharyngostrigea pipientis,* cysticerci.

Rana sylvatica: *Opalina obtrigonoidea, Trichomonas augusta, Haptophrya michiganensis, Brachycoelium salamandrae, Oxysomatium americanum,* larval acanthocephala.

ACKNOWLEDGMENTS

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A NEW EURYPTERA AND NOTES ON OTHER CERAMBYCIDAE
(COLEOPTERA)

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A key to species of *Euryptera*, along with description of a new species, description of the allotype of *Purpuricenus opacus* (Knull), and an addition to our fauna of a Cuban cerambycid, are presented.

Euryptera sabinoensis n. sp.

♀.—Rather robust, reddish brown throughout, elytra opaque, tips of mandibles, eyes, antennae, tips of femora, tibiae and tarsi dark brown to black. Densely clothed with reddish brown pubescence on dorsal surface, pubescence shorter beneath.

Head densely, minutely punctate on vertex, front rugose; antennae extending to middle of elytra when laid along side, scape stout, second segment about as long as broad, third nearly as long as scape, fourth shorter than fifth, sixth to tenth gradually shortening, eleventh as long as fifth.

Pronotum much wider than long, widest at base, strongly constricted at apex; sides rounded in front, then oblique to base; basal angles obtuse; basal margin sinuate, median lobe broad; disk convex, with slight median depression in front of scutellum and one on each side; surface densely finely punctate, punctures larger than those on head. Scutellum large, triangular.

Elytra near base wider than base of pronotum, widest back of middle; sides subparallel near base, then diverging and rounded in apical fourth to obliquely truncate apices, outer apical angles obtuse; disk of each elytron with two indistinct costae; surface densely, finely punctate, punctures well separated and larger than those on pronotum.

Abdomen beneath densely, minutely punctate.

Length 12.6 mm; width 3.8 mm.

Described from a single female collected in Sabino Canyon, Santa Catalina Mountains, Arizona, July 11, 1952, by D. J. and J. N. Knull. Holotype in collection of author.

This species is distinct from *E. ignita* (Schffr.) (1908) by the opaque elytra, finer punctuation of pronotum and rugose front. The following key will separate the known forms found north of Mexico.

1. Elytra in part black or dark brown.....(2)
   Elytra brown, or reddish brown.....(5)
2. Rather robust; pronotum yellowish brown.....*texanae* Knull
   Narrow elongate species; pronotum black or with black marking.....(3)
3. Punctures of elytra coarse, separated by nearly their own diameters at base.....*huachucae* Schffr.
   Punctures of elytra fine, dense, giving elytra a rugose appearance.....(4)
4. Elytra at least two-thirds black.....*lateralis lateralis* (Oliv.)
   Elytra two-thirds brownish yellow.....*lateralis flavatra* Blatch.
5. Scutellum and ventral surface dark brown, or black.....*cruneta* Martin
   Scutellum and ventral surface brown, or reddish brown.....(6)
6. Elytra opaque; punctures of pronotum fine, dense; tibiae dark.....*sabinoensis* n. sp.
   Elytra shining; punctures or pronotum coarse, distinct, well separated, tibiae in part light in color.....*ignita* (Schffr.)

Purpuricenus opacus (Knull)

A ♀ specimen of this species which was described by me as a *Tragidion* (1937) was captured in Jeff Davis Co., Texas, July 12, 1950 by D. J. and J. N. Knull. It should be placed in genus *Purpuricenus*. This specimen which I have designated as allotype has antennae extending to apical sixth of elytra. Elytra are reddish yellow and apical black area extends obliquely, sinuately from outer margin to one-third distance up suture. The elytra are margined with black at base.

Probatius umbraticus DuVal

This Cuban species should be added to our list. Specimens identified by G. B. Vogt were collected in Dade Co., Fla., June 9–16, 1949, May 20–25, 1950 and May 31, 1951, by H.F. Strohecker.

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Man and His Physical Universe. *Richard Wistar.* John Wiley and Sons, Inc. New York. 1953. xv+488 pp. $4.75.

A book written for science survey courses which attempts to show the unity of the physical sciences and their relations to everyday living. Topics are introduced through familiar examples from our workaday lives. There are numerous good illustrations. Chapter summaries and review questions are included at the ends of chapters.

EDWARD S. FOSTER, JR.

The Social Insects. *O. W. Richards.* Philosophical Library, Inc., New York. 1953. xiii+205 pp. $4.75.

While this book is intended for the general reader it is an excellent review of the social insects with emphasis on their behavior. Dr. Richards gives an up-to-date world wide approach to the subject with the following chapters: the Insect World, Solitary and Social Wasps, Varieties of Wasp Society, Solitary and Social Bees, Honey Bees, The Ants or Pismires, Social Parasites, The Termites, and Insect Societies. There are ten illustrations with fifty-one excellent photographs. This is an outstanding book for the reader of natural history.

E. W. HUFFMAN.

The Molecular Architecture of Plant Cell Walls. *R. D. Preston.* John Wiley & Sons, Inc., New York. 1952. xii+211 pp. $6.75.

The author presents the historical background of cell wall studies; discusses the various substances of which cell walls are constructed; illucidates the various physical methods used in investigating the structure of plant cell walls; reports on the detailed architecture of cell walls in a wide variety of plants; and attempts to interpret the growth of cell walls in terms of molecular structure. Much of the material is a report of original research done by the author and his associates, and is published in this book for the first time.

R. A. POPHAM

GREECE'S MAJOR PROBLEM: TOO MANY PEOPLE[1]

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The extremely high ratio between the population of Greece and its total productive area presents one of that nation's most crucial problems. The density per square mile of arable land was 580 in 1951 and for the total area 151 per square mile (Weaver and Lukerman, 1953). The latter figure represents about a tenfold increase since 1821 when it was 15. The high density for the country as a whole is more pronounced when one considers the fact that the major part of the country is mountainous and infertile.

The large Greek population is due to two main causes. The first cause is the high birth rate, exceeding that of any other European country with the exception of Yugoslavia and Poland. Improved sanitary and medical facilities have helped to reduce the mortality rate. In 1951 the birth rate per 1000 population was 26.1, an increase of 6.5 over 1941–45. The death rate was 10.7 as compared with 17.1 in 1941–45 (Population Index, 1952). The second was the influx of a large number of refugees—about 1.5 million—from Asia Minor in the period 1922–28 (Eddy, 1931). Although foreign minorities left the country during this period, newcomers exceeded them by far, the net gain having been 1.5 millions.

The combination of high birth rate and low mortality has resulted in a net surplus, which, in the pre-war period, amounted to about 85,000 people per annum and in 1951 to about 115,000. Thus, the annual rate of population increase is 1.4 to 1.5 percent. This annual increase exerts a strain on the economy of the nation, because it has been unable to provide full-time employment opportunities for the growing labor force. Here is a typical example. A village near Salonika with a population of 782 people in 1937, showed 314 births and only 161 deaths during the years 1937–1946 (Smothers *et al.*, 1948). The additional population had to be supported on the same amount of land as before, yet could not provide additional employment. This has been the case throughout the nation.

INTERNATIONAL TRADE

The increase in population has also altered international trade relations and the domestic political structure of the country. It has increased requirements for food, thus necessitating greater food imports. Politically new parties have arisen whose extreme slogans and sugar-coated measures have attracted the young men of the nation who lost faith in the older parties and even the nation's future. This unsteady course of Greek politics can be traced largely to a growing hostility between the landowners and the unemployed and underpaid city workers who feel that they are not an integral part of the nation. The city workers suffer the greatest deprivation, because they don't earn enough money to improve their economic and social status in the Greek community. The pressure of standards makes them want more material things than they can afford.

Why then does not the Greek peasant take the necessary preventive measures to slow the growth of the rural population? In the first place he looks upon his children as a guarantee against want in old age. The larger the family, the less the burden on each child who later must contribute to the support of the parents. Furthermore, the peasant considers more than four children, preferably boys, a sign of God's blessing and also proof of his virility.

[1]Published with the aid of a grant to The Ohio Journal of Science from the Charles F. Kettering Foundation.

Emigration has been an important factor in Greece's economy on account of the regular return flow of emigrant's remittances. The recipients of the remittances buy the goods and services which make living comfortable. Although providing a certain safety-valve for Greece's population, emigration has deprived the country of considerable productive forces. Only the old and very young stay behind. The Greek emigrants have found easy acceptance in the United States and in other countries. In 1951 the following countries received Greek immigrants: Israel, 133; Southern Rhodesia, 31; Union of South Africa, 124; United States, 1,449 (Demographic Yearbook, 1952).

Despite the efforts of the government to improve the domestic economy of the country, the Greek balance of trade is still unstable. Two main obstacles hamper an increase in exports. These are the fact that pre-war markets have been lost while the old markets are unable to buy as much as formerly. Greece was out of these markets during the war and has found it impossible to meet the new competition which displaced her.

To reduce her trade deficit, Greece was permitted under the European Recovery Program to enter into bilateral trade agreements with participating countries in the hope of increasing her exports. This, however, did not help Greece very much because exports of her major surplus products was not encouraged by other countries, particularly, wine to France, and tobacco and raisins to Great Britain. In 1949, the deficit was $332,000,000 (Greek Information Office, 1950). The deficit can be erased only by increasing the export of such products as tobacco, citrus fruits, olives, olive oil, wine and raisins, the demand for which is highly unstable.

Invisible earnings have been steadily decreasing because (1) of the assimilation of Greek emigrants in the countries where they were established and (2) the flight of Greek shipping capital abroad. Revenues from shipping have decreased because shipowners have changed their flags in order to avoid paying taxes to the Greek government. In 1949, 558 out of 985 Greek ships operated under foreign flags (Greek Information Office, 1950). Vessels which continue to sail under the Greek flag have under European Cooperation Administration (E.C.A.) and government pressure been paying their taxes to the Greek Treasury. This represents about 10 percent of the government's total expenditures.

AGRICULTURAL ADJUSTMENT

In 1947, the Greek government, with the help of E.C.A. initiated a broad program to promote productive agricultural projects whose main objectives were (1) to increase available land by draining swamps, (2) to protect crops from drought by extending the present irrigation system and (3) to check flood damage by erecting flood control devices. The real purpose behind the program is to increase the size of the farms. It has been estimated that the average size of a farm is about 8 acres (Smothers *et al.*, 1948). A larger farm would permit a full employment and greater production.

Prospects for increasing agricultural lands for the production of grain cereals are now favorable. According to one estimate, over 300,000 acres of the plains of Macedonia can be reclaimed through drainage, 680,000 acres in Thessaly and in Peloponnesus can be protected from floods and over 1,000,000 acres of dry lands throughout the country can be irrigated (Greek Information Office, 1950).

E.C.A's mission to Greece has been mainly responsible for the improvements that were registered by Greek agriculture from 1948–1952. The 1.5 billion dollars which the American taxpayer has spent on the rehabilitation of Greece since the end of the war seems a great deal of money to invest in a Balkan country the size of the State of New York. But here certainly was a worthwhile investment. The aid shows encouraging results. Yet these results might have been more decisive and promising had Grece been in the same position as many of the European

countries at the conclusion of the war, that is, if Greece had not been involved in Civil War.

In estimating the amount of progress made in the rehabilitation of agriculture, one finds a return to pre-war production trends in some agricultural products —wheat, barley, corn—and some encouraging results in the cultivation of new commodities, namely, cotton and rice. For example, the production of grain has reached its pre-war level of 770 thousand metric tons, while that of cotton has increased from 76,000 to 112,000 bales. More than 100,000 acres of land have been added to the existing total through the draining of swamps and the extension of the irrigation system (Greek Information Office, 1950).

However, the most important consequence of American Aid to Greece has been the change that it caused in the attitude of the peasent concerning his role in the economy of the country. The aid convinced him that in his hands are the tools for a better life. On the other hand, the future of the Greek agriculture does not depend on him alone, but also in the trade relations among the countries of the world. If international trade were accelerated, Greece could develop the crops best adapted to her soil and climate, such as tobacco growing, fruit growing, vine-dressing and raisin cultivation. But, if the present character of international trade continues, Greece will be compelled to increase her production of expensive grains in order to feed her constantly growing population.

THE INDUSTRIAL SITUATION

The Greek government has tried to improve the lot of the urban labor force by using part of American aid to develop industry. The most important phase of the industrialization program has been the construction of hydro-electric plants. Funds for these plants were assured through the Marshall Plan plus dollars from Italian reparations. The impact which the building of these power plants will have upon the entire Greek economy can be appreciated when we realize that at the present time electric power in Greece does not exceed 270,000 kw of which 150,000 are distributed in the Athens-Piraeus area. With the completion of the hydro-electric plants, available electric power will be almost doubled (Xydis, 1950).

There is no doubt that income from farming in Greece would be effectively supplemented by the availability of cheap and plentiful electric power. Rural home industries could be materially helped by the supply of electricity to many districts, especially in Macedonia. Thus, the first objective to be aimed at in the electrification of the country must be the use of electricity to increase the farmer's income to the maximum, that is, to about two dollars per day, in contrast with the current 75 cents or little more (U.N. Statistical Papers, Series E, No. 1, 1950 and No. 3, 1951). Otherwise the best possible foundation will not be laid for the development of industry.

SUMMARY

Greece's great problem is under-employment. The country's main economic activity, agriculture, is hampered not only because of too small farms but also by the lack of good roads, arable land and water supply. Many people are underfed because after the harvest is in, there is slight opportunity for them to augment their income until the following spring when they begin to farm again. In such circumstances, better than outright technical assistance would be the instructing of the people in ways to utilize more efficiently than they do their produce and also to encourage them to develop their own technology.

Despite the conscientious effort of the Greek government to improve the agricultural and industrial economy of the country in order to provide more employment opportunities and to increase the employment period, one should not look for an immediate dramatic and substantial rise in the Greek standard of living.

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Advanced Mathematics in Physics and Engineering. *Arthur Bronwell.* McGraw-Hill Book Co., Inc., New York. First edition, 1953. xvi+475 pp. $6.00.

Here is a text for senior or graduate students in engineering and physics. Besides presenting the advanced mathematics which constitute the principal analytical methods of physics and engineering the book seeks to demonstrate how many of the basic laws in these fields can be expressed in very general form by a few fundamental mathematical formulations, which in turn form the springboard for the development of vast areas of applications. Solutions of typical problems are included to provide concrete examples of the mathematical methods.

A STUDY OF THE EFFECT OF HETEROZYGOSIS ON LITTER SIZE IN THE MOUSE *MUS MUSCULUS*

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The experiment reported here was undertaken to test whether litter size in the mouse *Mus musculus* is a trait which is exclusively determined by the mother's heredity and condition, or whether perhaps the heredity of the zygotes, aside from homozygosity for lethal genes, might also have some effect. Since any increase in litter size due to the embryos' heredity might be slight, care was taken to design an experiment with maximum possible sensitivity for this effect. The method used was to compare litter sizes of sister mice from inbred strains one of which was mated to her brother while the other was out-crossed. Appropriate statistical analysis of the data secured showed a significant increase in litter size where the young born were heterozygous. It seems justifiable therefore to conclude that heterozygosity of the young can increase litter size.

The character "litter size" may be defined as the number of young born, whether alive or dead. It is not a simple character but a function of at least three variables, viz., fecundity, number of eggs fertilized, and amount of pre-natal mortality. Fecundity, the number of eggs liberated by the ovaries at an ovulation, naturally sets the upper limit for a possible litter size. The number of eggs ovulated as measured by the number of corpora lutea has been found to increase with parity (the number of times the mother has borne a litter) and maternal age (MacDowell and Lord, 1925; MacDowell, et al., 1929). The increase is rapid from the first to the third litter but slow afterwards, but there is no decline until the eleventh litter.

The other two variables limit the possible litter size. Thus eggs unfertilized and embryos dying before birth will not contribute to the actual litter size attained. Partial or complete sterility in the male naturally will increase the number of eggs unfertilized. The failure of embryos coming to term may be due to various causes. Aside from homozygosity for lethal genes which would not operate in inbred lines, a possible cause could be homozygosity for genes impairing vigor. Under unfavorable conditions in the uterus of a mother in poor health, embryos homozygous for such sublethal genes might fail to complete development. Such genes might be carried by inbred lines. Maternal influences tending to decrease viability of offspring would be such conditions of the mother as would affect her ability to carry and nourish her young. Since litter size regularly decreases in females after the first four or so litters while the number of eggs ovulated does not show a decrease until the eleventh litter, the decrease in litter size is mainly due to an increase in pre-natal mortality (Grüneberg, 1952). Fekete (1947) has found in mice of the DBA strain a high pre-natal mortality (41.7%), and has shown experimentally that this is due to the unfavorable environment of the DBA uterus. An earlier investigation of normal strains which compared the number of living young born with the number of corpora lutea formed during the corresponding pregnancies showed a pre-natal mortality of 33.9 percent (MacDowell, 1924). A change in diet from rolled oats to "Fox Chow" has raised mean litter size from 4.83 ± 0.04 to 5.68 ± 0.04 (Bittner, 1936). Presumably the latter diet increased fecundity or decreased pre-natal mortality or perhaps did both.

Some work which bears on the question whether litter size is determined exclusively by the mother has been reported in the literature. C. V. Green (1931)

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found that litter size was determined wholly by the female in a mouse species cross (*Mus musculus* × *Mus bactrianus*). Wright (1922) in a careful study of the effects of cross-breeding between highly inbred families of guinea pigs also came to the conclusion that the heredity of the young had no effect on litter size. He did not note a slight effect of the heterozygosis of the young in an increase of the percentage born alive. The effect however was insignificant and perhaps due to chance.

EXPERIMENTAL METHODS AND MATERIALS

Description of Mice Used

Two highly inbred strains of mice were used in this experiment, the BALB/c and the DBA/2 strains. The BALB/c mice were descendants of a single pair obtained by E. L. Green from the Jackson Memorial Laboratory in 1937 and were in the 78th to the 79th generations of brother × sister inbreeding. The DBA/2 mice were descendants of one pair of mice received by Dr. Green from Dr. L. W. Law of the National Cancer Institute in 1948 and were in the 36th to 38th generations of brother × sister inbreeding.

The Experimental Design

Originally it was planned to investigate in this experiment the effect of the female and the effect of the male, as well as the effect of the heterozygosity of the young on litter size. Therefore a factorial experiment was designed which incorporated all possible combinations of the experimental material. Let B, B1, B2 be BALB/c strain mice, brother and two sisters, and similarly let D, D1, D2 stand for DBA/2 mice. Four kinds of matings were made: B × B1; B × D2; D × D1; D × B2. The first two matings needed only one cage since the same male was used. For the same reason, one cage sufficed for the last two matings. The young born in each cage from each type of mating were easily distinguished, for the cross-bred young were always brown agouti while the inbred young were always either albino or dilute brown non-agouti. Thus mistakes in classification if made at birth were rectified later when the fur appeared. The four matings described constituted one block. Ten replications (ten blocks) were set up. The animals selected for each block were always approximately the same age and were kept during the experiment under the same environmental conditions. The cages were inspected at least weekly for pregnant mice, which however were not removed from the mating cages. They were left with the males in order that they might mate at the post-partum oestrus and thus have their next litters with less delay. As soon as the young were furred sufficiently to be positively identified as to origin, they were killed. Cages with pregnant females were inspected daily for litters born and the pertinent data recorded. The experiment was begun November 15, 1950, and was terminated on June 6, 1951.

RESULTS OF THE EXPERIMENT

Data Obtained

Matings involving the DBA/2 females produced scanty results. Five of them produced no young at all during the time of the experiment. Since the data secured from the DBA/2 females were incomplete and useless for analysis, they are not given here. The data from the BALB/c females were complete enough for analysis and are given in table 1, where sizes of litter are recorded for each pair of mice compared. Each mouse is identified by her ledger number and in each case the female bred to her own brother is placed first. The litters of the two differently mated sister mice were matched according to date of birth rather than according to the number of the litter in the series. An x marks the absence of one litter of a pair. This method of matching was used in order to make the

litters as comparable as possible since mice born at different times might have been carried under somewhat different environmental conditions and the number of eggs ovulated might have differed in mice of different age. Of course it was impossible to match litters exactly as to date of birth; the mean difference between the dates for the matched litters was 5.8 ± 0.68 days. It is possible that some litters were absent because all the young died before birth, or were eaten immediately after birth, or the female failed to conceive for a number of ovulations.

Inspection of these data shows that the matings involving the BALB/c females produced sufficient offspring to allow a reliable statistical analysis. In no case did a BALB/c female produce fewer than two litters.

Results of the Statistical Analysis

As mentioned, the experiment was planned as a factorial experiment, and therefore embodied all possible combinations of the material. Since the matings involving the DBA/2 females produced insufficient results, it was necessary

TABLE 1

Size of litter for BALB/c females. First mouse in pair inbred; second out-crossed. An x means that a matching litter was absent.

MOUSE	LITTER SIZE						
02	5–1*	2	6	3	6	2	x
03	9	11	5	8	6	9	10
09	6	5	(1)#	4	2	6	
10	7	8	9	x	5	x	
36	7	6	8	x			
37	9	10	7	10			
53	1	x	4	4			
54	4	5	8	4			
58	x	12	x	10	3		
57	1	8	8	9	x		
59	2	x	4	x			
60	8	11–1*	6	8			
86	5	2	2				
87	9	x	5				
95	2	4	5				
96	2	9	7				
101	3	6	3				
102	7	(2)#	3				
104	7	9					
105	4	4					

*Refers to the number of mice found dead in the litter.
#Data unusable because of unknown origin or depletion.

to modify the method of statistical analysis originally planned. The analysis of variance with multiple classification of the material was used to test the significance of the differences between litter sizes obtained from the BALB/c females only, as well as to test whether the matched litters for the different pairs of females constituted a homogeneous population. Thus one original object of the experiment was attained, namely, to test for significant differences caused by heterozygosity of the young.

Litter size is considered to be a variable distributed according to the Poisson distribution. This view is based on the idea that the birth of any particular individual mouse is an event whose probability is uniform but ordinarily rather small and that the size of a litter could theoretically at least be quite large. Thus the occurrence of 0, 1, 2, 3, . . . mice in a litter would follow the Poisson distribution, in which the variance is equal to the mean and therefore is perfectly correlated

with it. The analysis of variance cannot be used with raw data where there is correlation between means and variances (Bartlett, 1947). A transformation of the data can be used which will eliminate the correlation. The one suitable here is the transformation to $\sqrt{x} + \sqrt{x+1}$ where x is the raw datum (Freeman and Tukey, 1950). This transformation was performed on the data of this experiment and the statistical analysis was applied to the transformed data. In every case of course only litters which could be matched were used as the source of data. Table 2 gives the transformed data for the litter sizes where the mother was a BALB/c female. The differences between the values for the inbred and outbred mice in each pair is also recorded. The first litter in any pair was the one inbred. A glance at the differences shows a pronounced trend in a positive direction, favoring the litters where the young were heterozygous.

TABLE 2

Litter sizes for BALB/c females transformed to $\sqrt{x} + \sqrt{x+1}$, *where x is observed litter size. First mouse in pair inbred; second out-crossed.*

Mouse		Paired Litters						Mean Differ.
02		4.7	3.1	5.1	3.7	5.1	3.1	
03		6.2	6.8	4.7	5.8	5.1	6.2	
	Diff.	1.5	3.7	−0.4	2.1	0	3.1	1.64
09		5.1	4.7	3.1				
10		5.5	5.8	4.7				
	Diff.	0.4	1.1	1.6				1.02
36		5.5	5.1	5.8				
37		6.2	6.5	5.5				
	Diff.	0.7	1.4	−0.3				0.57
53		2.4	4.2	4.2				
54		4.2	5.8	4.2				
	Diff.	1.8	1.6	0				1.14
58		7.1	6.5					
57		5.8	6.2					
	Diff.	−1.3	−0.3					−0.78
59		3.1	4.2					
60		5.8	5.1					
	Diff.	2.7	0.9					1.77
86		4.7	3.1					
87		6.2	4.7					
	Diff.	1.5	1.6					1.51
95		3.1	4.2	4.7				
96		3.1	6.2	5.5				
	Diff.	0	2.0	0.8				0.91
101		3.7	3.7					
102		5.5	3.7					
	Diff.	1.8	0					0.87
104		5.5	6.2					
105		4.2	4.2					
	Diff.	−1.3	−2.0					−1.58

Once the values for the litter sizes had been transformed and the differences between matched litters calculated, the analysis of variance was used to test whether the differences were actually significant and not merely due to the chances of random sampling. Table 3 summarizes the result of the statistical test. The calculated ratio of the mean squares for "between matings" and "interaction," F = 12.15, is greater than the critical value at the 1 percent level of significance, F = 7.68. Therefore rejection of the null hypothesis of no difference is indicated.

Once it was established that there was an over-all significant difference in the sizes of the two different kinds of litters, it became of interest to test whether the

matched litters for the different pairs of females constituted a homogeneous population. The analysis of variance summarized in table 3 provided information which allowed testing this question. It will be seen that the value of F calculated (the quotient of the mean squares for "between pairs of females" and "between pairs of matched litters from the same female pairs"), 2.75, is about at the critical value for 3.7 percent probability. Therefore there is some reason to think that the litters of the different pairs of females did not constitute an entirely homogeneous population. In the cases of the female pairs 58–57 and 104–105, the inbred sisters had the larger litter sizes. These pairs injected some heterogeneity into the otherwise homogeneous population of litter sizes.

DISCUSSION

This experiment detected a significant increase in litter size which may be ascribable to the heterozygosity of the young. The use of inbred lines enabled the experiment to be quite sensitive to this effect. Litters of a sister inbred differed from the litters of her sister outcrossed only in the fact that the latter contained mice that were heterozygous for at least some loci whereas the former contained mice that were nearly completely homozygous. Thus the significant difference in litter sizes seems ascribable to the single differential factor of heterozygosity.

TABLE 3

Analysis of Variance

SOURCE OF VARIATION	DEGREES OF FREEDOM	MEAN SQUARE	F
Between matings	1	10.542	12.15
Between pairs of litters	27		
Between pairs of females	9	2.239	2.75
Between matched pairs of litters from same female pairs	18	0.813	
Interaction	27	0.868	

After this experiment was begun, O. N. Eaton in a preliminary report (1950) on extensive experiments made to test for the effect of heterozygosity of dams and litters on characteristics of offspring in mice, reported that greater litter size in certain cases seemed to be due to greater survival among heterozygous mice.

A similar effect on litter size has been found in rabbits. Gregory (1932) observed larger litters from crossbred does than from inbred does. He ascribed this effect to a slightly higher vitality of the hybrid young during the gestation period.

The same effect has been detected in chickens. Byerly (1930) presented evidence which showed that mortality of hybrid chicks in the eggs is less than is the case when the eggs contain embryos resulting from matings between cocks and hens of the same strain.

It has long been known that crosses between different strains often result in a considerable increase in vigor, as shown by size and productivity excelling that of either of the parent strains. This phenomenon of "heterosis" has been exploited by those dealing with hybrid corn. Wright (1922) in his study of the effects of crossbreeding on characters fixed in inbred lines of guineapigs found such heterosis strikingly shown, for example, in increased size of litters from hybrid females, increased resistance to tuberculosis, etc. It is not surprising then that this hybrid vigor can be shown even in utero and that it is manifest

in larger numbers of young coming to birth. It is the same phenomenon detected at an early period of development.

Why then did not Green (1931) or Wright (1922) secure evidence for the effect of hybrid vigor operating already in utero? Wright himself said that *a priori* one would expect the inherent vigor of the young in utero to count for something. Litter size is affected by many influences of which the more important known ones have been summarized in the introduction. The effect of hybrid vigor in the young may not be as decisive in influencing litter size as the other factors. Its effect might easily be lost in the random interplay of the other factors. Thus one might expect to detect the effect of hybrid vigor only if the animals used in the experiment are as uniform as possible and subjected to as uniform a treatment as possible except for the one variable whose effect is being tested. It may be that in Green's experiments this ideal was not attained. In the case of Wright's guinea pigs, the experiments extended over four years when environmental conditions were exceptionally varied. He applied corrections for seasonal fluctuations of conditions but perhaps these corrections were not precise enough to avoid loss of the hybrid vigor effect. Another factor may be the small sizes of guinea pig litters. Wright states that litters of from two to four are most common, with litters of more than six quite rare. It is conceivable that hybrid vigor affecting litter size might more easily fail to be detected when the variable has so small a range.

As a conclusion to this study, a possible genetic explanation of the increase in litter size due to heterozygosity will be given. It is modeled on the genetic explanation advanced by Wright (1922) to explain hybrid vigor in general. Dominant deleterious mutations are eliminated rapidly in a species in nature. Various recessive deleterious mutations however can be carried in heterozygous condition and can accumulate. Inbreeding produces homozygosis which is practically complete after the fifteenth generation of brother-sister mating. Thus recessive factors become homozygous and become effective in the inbred strain. Any recessive lethal factors will destroy their possessors but the recessives which are less deleterious can become fixed in the strain and can induce a hereditary lowering of vigor. One sign of this decline in vigor can be failure of some young to complete growth in the uterus and thus fail to be born. Among several inbred strains it is likely that homozygosis of recessive deleterious factors is attained at different loci. Thus crossing between these strains results in an improvement in vigor and litter size because each strain supplies dominant factors lacking in the other which prevent expression of their recessive deleterious alleles.

SUMMARY

A study of the possible effect of heterozygosity of the young or litter size was made. Inbred female mice were bred to their brothers while sisters were outcrossed. Data secured from matched litters of BALB/c mice were sufficient to support a valid statistical analysis. The mean litter size in litters where the young were inbred was 4.82 mice while the mean litter size in litters where the young were hybrids was 6.82 mice. On statistical analysis, the difference of two mice was found to be significant. This difference is ascribable to heterozygosity of offspring which caused greater litter size. The population was not quite uniform in this effect. An explanation is offered of a possible genetic basis for the effect.

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Introduction to Mathematics. *Stephen C. Kleene.* D. Van Nostrand Co., Inc., New York. First edition, 1952. x+550 pp. $8.75.

The book is meant as a text book for first year graduate students in mathematics or others at that level of mathematical facility. Its aim, as stated by the author: ". . . is to provide a connected introduction to the subjects of mathematical logic and recursive functions in particular, and to the newer foundational investigations in general." This book is also being published in Holland as a volume in *Bibliotheca Mathematica.*

The Algae of Illinois. *Lewis H. Tiffany* and *Max E. Britton.* University of Chicago Press, Chicago. First edition, 1952. xiv+407 pp. $10.00.

This well illustrated volume (over 1200 line drawings in 108 plates) will be useful far beyond the boundaries of Illinois. The indicated procedure for identifying an unknown species is as follows: The phyla are delimited in the Introduction, while the classes are indicated by a short synoptic key and text descriptions. Orders and families are adequately separated by concise descriptions. Genera and species receive detailed analyses, with keys to the genera in each class and keys to the species in each genus. The final determination should be checked with the description and drawings in the book as well as authentic reference material whenever possible.

Foundations of Biology—A Laboratory Handbook. *Gardiner B. Moment* and *Helen V. Crouse.* Appleton-Century-Crofts, Inc., New York. 1953. v+282 pp., paper-bound. $2.50.

Although meant for use in a biology laboratory course this volume is called a handbook rather than a manual by its authors. Probably this is because the 132 pages of descriptive material are separate from the workbook unit of 150 pages. The authors have consciously tried to avoid the habitual approaches to their subject and have not hesitated to include material from the newer areas of biological science. Their final exercise, "Evolution, Comparative Anatomy and Systematic Serology," is an example of the unique approach.

(F. W. F.)

Elementary Statistics with Application in Medicine. *Frederick E. Croxton.* Printice-Hall, Inc., N. Y. 1953. vii+376 pp. $7.50.

This book covers all of the standard topics and methods of elementary statistics with apt examples from the medical literature. Each chapter is prefaced by a list of symbols with definitions used in the chapter. Fourteen tables at the end make this one book a self-contained library for the most common statistical applications.

E. L. GREEN

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